Kainic acid: neurotoxic effects after intraocular injection

Robert Schwarcz and Joseph T. Coyle

Intraocular injection of 120 nmol. of kainic acid, a powerful glutamate receptor agonist, induces a marked degeneration of cells in the inner nuclear layer of the retina. Within 2 hours after injection there is a significant decrement in the specific activities of tyrosine hydroxylase, choline acetyltransferase, and glutamic acid decarboxylase; by 48 hours after injection there is nearly a complete loss in the presynaptic neurochemical markers for the cholinergic and GABAergic neurons. The dopaminergic neurons, as assessed by activity of tyrosine hydroxylase and concentration of endogenous dopamine, are reduced only 50% by the kainic acid treatment. Although basal adenylate cyclase activity is unaffected by kainic acid, there is a 90 percent reduction in the activating effects of dopamine on adenylate cyclase in the kainic acid-treated retina.

Key words: acetylcholine, 7-aminobutyric acid, dopamine, dopamine-sensitive adenylate cyclase, glutamate receptors, kainic acid, retinal degeneration.

The parenteral administration of monosodium glutamate to immature mice produces a degeneration of several cell types in the inner retina while sparing cells in the outer retina, particularly the receptors.1 Dicarboxylic amino acids including glutamate have long been known to exert excitatory effects on most neurons in the central nervous system.2 Olney and associates3 have demonstrated a close correspondence in the retina between the neurotoxic properties and the neuroexcitatory actions of several amino acids structurally related to glutamate. Thus they have hypothesized that the neurotoxicity of these agents may be due to an excessive stimulation of receptors for glutamate on retinal neurons. Recently, a heterocyclic rigid analogue of glutamate, kainic acid, has been shown to be considerably more potent as a neuroexcitant than glutamate itself.4 Although the systemic administration of kainic acid exerts neurotoxic effects on central nervous system (CNS) neurons, its marked toxicity resulting in seizures and death has limited its experimental use.5

With the exception of studies concerning the effects of systemic administration of monosodium glutamate on enzymes that
metabolize glutamic acid in the retina, there has been little investigation of the neurochemical consequences of such retinal lesions. Considerable evidence has accrued indicating that cholinergic, \(7'8\)-amino-nootropic, and dopaminergic neurons contribute to that population of interneurons in the retina designated as amacrine cells. Since the amacrine cells are particularly sensitive to the glutamate-induced degeneration, it would be of interest to determine whether these specific neuronal types are also affected. Accordingly, we have examined the effects in the chick retina of direct intraocular injection of kainic acid on the histologic characteristics and neurochemical markers for the cholinergic, GABAergic, and dopaminergic neurons in the retina.

**Methods**

**Preparation of tissues.** Young chicks (2 days old) were obtained from Truslow Farms (Chester-town, Md.). Kainic acid (Sigma Chemical Co., St. Louis, Mo.) was dissolved in sterile normal saline solution and titrated to pH 7.4; 120 nmol of the kainate in a volume of 5 \(\mu\)l was injected intraocularly with a Hamilton microsyringe. Controls received intraocular injection of 120 \(\mu\)l of \(\alpha\)-methylaspartate, an inactive analogue of glutamate. At various times after injection the chicks were killed by decapitation; their eyes were removed and immediately immersed in ice-cold 0.32 M sucrose buffered with 0.01 M Tris-acetate, pH 7.4; and the retinas were isolated by gentle dissection from the choroid.

**Enzyme assays.** The retinas were homogenized in 10 volumes (w/v) of 0.05M Tris-HCl, pH 7.4, containing 0.2% (v/v) Triton X-100. A 50 \(\mu\)l portion of the homogenate was assayed for tyrosine hydroxylase activity at a final concentration of L-tyrosine of 50 \(\mu\)M according to the method of Coyle and Oderfeld-Nowak. In the presence of 0.5 \(\mu\)M \(\left[{\text{3}}\text{H}\right]\text{choline or 1 \(\mu\)M [\(\text{3}\)H]GABA as described by Coyle and Enna. Uptake was terminated by placing the tubes in an ice-water bath; and they were immediately centrifuged at 10,000 \(\times\) g for 10 minutes at 5° C. The radioactive substrate accumulated in the pellet was determined by liquid scintillation spectrometry.

**Protein.** Protein was determined by the method by Lowry and associates, using bovine serum albumin as the standard.

**Histologic studies.** After decapitation the eyes were removed and placed in buffered formaldehyde fixative. The whole eye was embedded in paraffin and sectioned at 15 \(\mu\)m intervals; the sections were stained with cresyl violet.

For the measurement of the levels of endogenous GABA and acetylcholine, retinas were homogenized in 20 volumes of formic acid–acetone (15:85) and then centrifuged. A 5 \(\mu\)l portion of the supernatant fluid was dried down in a vacuum centrifuge and the GABA was measured by the fluorometric enzymatic assay of Graham and Aprison. A 50 \(\mu\)l portion of the supernatant fluid was assayed for acetylcholine by the radiometric enzymatic assay of Goldberg and McCaman. Adenylate cyclase activity. Retinas were homogenized in 20 volumes (w/v) of ice-cold 2 mM Tris-acetate, pH 7.4, containing 2 mM ethylene glycol-bis(\(\beta\)-aminoethyl ether) N,N'-tetraacetic acid (EGTA). Incubation was initiated by adding 50 \(\mu\)l of the homogenate to 250 \(\mu\)l of 80 mM Tris-acetate buffer, pH 7.4, containing 4 mM MgSO\(_4\), 10 mM theophylline, 0.2 mM EGTA, and 0.5 mM adenosine triphosphate (ATP) according to the method of Kebabian and associates. Incubations were terminated after 2.5 minutes by heating the reaction mixture for 10 minutes at 95° C. After centrifugation, a 25 \(\mu\)l portion of the supernatant was taken for determination of adenosine 3',5'-cyclic monophosphate (cyclic AMP) by the competitive binding assay. The kinetic and pharmacologic characteristics of the dopamine-sensitive adenylate cyclase in the chick retina have been described previously.

**Uptake studies.** Retinas were homogenized in 10 volumes of 0.3M sucrose buffered with 50 mM Tris-HCl, pH 7.4, in a smooth glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 1,000 \(\times\) g for 10 minutes at 5° C. to remove nuclei and cell fragments; and the supernatant was centrifuged at 20,000 \(\times\) g for 30 minutes to isolate the Ps fraction. The pellet was gently resuspended in an equal volume of the buffered sucrose. Portions of the resuspended homogenate (50 to 100 \(\mu\)l) were incubated in Krebs phosphate buffer for 4 minutes in the presence of 0.5 \(\mu\)M \(\left[{\text{3}}\text{H}\right]\text{choline or 1 \(\mu\)M [\(\text{3}\)H]GABA as described by Coyle and Enna. Uptake was terminated by placing the tubes in an ice-water bath; and they were immediately centrifuged at 10,000 \(\times\) g for 10 minutes at 5° C. The radioactive substrate accumulated in the pellet was determined by liquid scintillation spectrometry.

**Histologic studies.** After decapitation the eyes were removed and placed in buffered formaldehyde fixative. The whole eye was embedded in paraffin and sectioned at 15 \(\mu\)m intervals; the sections were stained with cresyl violet.

Table 1. Effects of kainic acid on neurotransmitter parameters of chick retina

<table>
<thead>
<tr>
<th>Neurochemical parameters</th>
<th>Units</th>
<th>Control</th>
<th>Kainate</th>
<th>Δ%†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholinergic:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Choline acetyltransferase</td>
<td>nmol./mg./hr.</td>
<td>7.2 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>-80</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>pmol./mg.</td>
<td>20.7 ± 1.0</td>
<td>4.4 ± 0.6</td>
<td>-79</td>
</tr>
<tr>
<td>Choline uptake</td>
<td>pmol./mg./4 min.</td>
<td>0.48 ± 0.05</td>
<td>0.15 ± 0.02</td>
<td>-67</td>
</tr>
<tr>
<td>GABAergic:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate decarboxylase</td>
<td>nmol./mg./hr.</td>
<td>0.87 ± 0.04</td>
<td>0.07 ± 0.01</td>
<td>-92</td>
</tr>
<tr>
<td>GABA</td>
<td>nmol./mg.</td>
<td>2.64 ± 0.11</td>
<td>0.26 ± 0.02</td>
<td>-90</td>
</tr>
<tr>
<td>GABA uptake</td>
<td>pmol./mg./4 min.</td>
<td>5.7 ± 0.5</td>
<td>0.4 ± 0.1</td>
<td>-93</td>
</tr>
<tr>
<td>Dopaminergic:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine hydroxylase</td>
<td>pmol./mg./hr.</td>
<td>42 ± 3</td>
<td>23 ± 3</td>
<td>-45</td>
</tr>
<tr>
<td>Dopamine</td>
<td>pmol./mg.</td>
<td>1.46 ± 0.20</td>
<td>0.66 ± 0.14</td>
<td>-55</td>
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</tbody>
</table>

*Forty-eight hours after the intraocular injection of 120 nmol. of kainate, retinas were assayed for various neurochemical parameters (as described in the methods section) and compared to uninjected retinas. The values are a mean ± S.E.M. of at least five separate preparations.

†p < 0.01.

Results

Time course of effects of kainic acid on neurotransmitter synthesizing enzymes in retina. After the injection of 120 nmol. of kainic acid there was a very rapid fall in the activities of the enzymes in the biosynthetic pathway for dopamine, acetylcholine, and GABA (Fig. 1). Two hours after injection the specific activities of choline acetyltransferase and glutamic acid decarboxylase were reduced by 50%, and 48 hours after injection the loss in the activity of these enzymes was nearly complete. Although the specific activity of tyrosine hydroxylase decreased by 35 percent 2 hours after injection, the activity of this enzyme remained at about 50 percent of control at 18 and 96 hours after injection. These changes in enzyme activity were not caused by the injection procedure itself since the vehicle and α-methylaspartate (50 μg), an inactive analogue of glutamate,3 were without effect on the neurotransmitter synthesizing enzymes. A dose-response curve (not shown) indicated that threshold toxic effects of kainic acid occur with 12 nmol., that maximal effects of kainic acid occurred with 120 nanomol., and that doses in excess of 120 nmol. did not further affect enzyme activity.
Fig. 2. Vertical sections through chick retina prepared 48 hours after intraocular injection of 120 nmol. of α-methylaspartate (A) or 120 nmol. of kainic acid (B). Sections (25 μm) are stained with cresyl violet. Photomicrographs were obtained in the region of the retina approximately 2.0 mm. from the fovea. Bar = 25 μm.

Effect of kainic acid on several neurochemical parameters of retina. Since many factors other than neuronal degeneration could alter the activity of neurotransmitter synthesizing enzymes in the retina, we examined several other neurochemical parameters 48 hours after the injection of 120 nmol. of kainic acid (Table I). In association with the profound fall in the specific activity of glutamic acid decarboxylase, there was a 90 percent decrease in the levels of endogenous GABA and of the activity of the high-affinity transport process for GABA in retinal homogenates. Similarly, there was a proportional reduction in the activity of choline acetyltransferase and its product, endogenous acetylcholine. The high-affinity transport process for choline, which is associated with cholinergic neurons, was also reduced 83 percent. The 50 percent decrement in the specific activity of tyrosine hydroxylase in the injected retina was similar in degree to the reduction in the concentration of its end product, dopamine.

The postsynaptic action of dopamine is mediated by a specific adenylate cyclase in brain and retina. Accordingly, we examined the effects of kainic acid treatment on adenylate cyclase activity in ret-
inal homogenates (Table II). The specific activity of adenylate cyclase in the absence of agonist was unchanged in the injected retina. However, the activation of adenylate cyclase by dopamine (50 μM) was reduced by 90 percent in the injected retina.

Histology of kainic acid lesion of retina.
To evaluate the effects of the injection procedure, sections through the whole eyes from chicks that had received intraocular infusions of 120 nmol of kainic acid or of the inactive analogue α-methylaspartate were examined. There was a modest amount of inflammatory cell infiltrates in the anterior chamber, around the zonular fibers near the injection site and adherent to the anterior aspects of the vitreous. Whereas the retinas of the α-methylaspas- tarate–treated chicks were indistinguishable from those of controls, the retinas of the kainate-treated animals exhibited marked and characteristic changes (Fig. 2).

By 48 hours after injection the kainate lesion involved the entire retina from the optic disc to the ora serrata. There was a 30 percent decrease in the overall thickness of the retina, which primarily reflected a 50 percent decrease in the depth of the inner nuclear layer. The contraction of the inner nuclear layer was due to a marked reduction in the cell number; many of the remaining cells exhibited pyknotic and distorted nuclei. The inner plexiform layer had a "Swiss-cheese" appearance with large vacuoles that were particularly evident in osmicated sections. The number of ganglion cells appeared to be slightly reduced. The outer layers of the retina were also affected with vacuolization of the receptor processes and disorganization of the outer nuclear layer; notably the outer limiting membrane appeared intact. By day 5, the loss of cells in the inner nuclear layer was nearly complete (90 percent), whereas the morphologic characteristics of the receptors, inner plexiform layer, and ganglion cell layer remained similar to those found at the previous stage of examination.

<table>
<thead>
<tr>
<th>Homogenates</th>
<th>Basal</th>
<th>Dopamine (50 μM)</th>
<th>Stimulation</th>
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<tbody>
<tr>
<td>Control</td>
<td>10.1 ± 0.2</td>
<td>21.7 ± 0.7 f</td>
<td>11.6 ± 0.6</td>
</tr>
<tr>
<td>Kainate injected</td>
<td>9.7 ± 0.2</td>
<td>11.2 ± 0.3 t</td>
<td>1.5 ± 0.3 t</td>
</tr>
</tbody>
</table>

Thus, although kainate had widespread effects in all layers of the retina, severe cell loss was confined to the inner nuclear layer.

Discussion
The intraocular injection of small amounts of kainic acid induced a rapid degeneration of cells and processes in the inner aspects of the retina. The characteristics of this retinal degeneration are similar to those described by Olney,1 wherein immature mice were administered high doses of monosodium glutamate by a systemic route. The demonstration by Olney and associates3 of the correlation between the neuroexcitatory and neurotoxic effects of several analogues of glutamate suggested that neurotoxicity may be due to overstimulation of glutamate receptors on retinal neurons. The findings of the present study that kainic acid, electrophysiologically one of the most potent agonists at glutamate receptors,4 produces similar neuropathologic changes in the retina lends further support to the hypothesis that neurotoxicity is related to excessive neuronal depolarization. Such pathologic changes induced by glutamate receptor stimulation are particularly significant in light of the growing evidence that glutamate may be involved in neurotransmission between photoreceptor, horizontal, and/or bipolar cells.6

With the advent of specific histochemical and microchemical techniques, it has recently become possible to characterize the distribution and localization of the processes involved in neurotransmission in the

Table II. Effect of kainate on adenylate cyclase activity (pmol./mg./2.5 min.) in the chick retina*
The high activity of glutamic acid decarboxylase and high concentration of GABA in the inner nuclear and inner plexiform layers as well as the ability of certain amacrine cells to take up radio-labeled GABA strongly suggests that GABA may be a neurotransmitter in a subpopulation of amacrine cells. Similarly, the high activity of choline acetyltransferase in the inner nuclear and inner plexiform layer and the dense staining for acetylcholinesterase on certain amacrine cells suggest that some of these cells are cholinergic. In addition, a small number of amacrine cells have been shown by histofluorescent microscopy to contain dopamine; the fact that dopamine can be synthesized, taken up, released, and then interacts with appropriate postsynaptic receptors in the retina provides compelling evidence that dopamine is a retinal neurotransmitter.

The kainic acid lesion results in a considerable loss of cells in the inner nuclear layer where the bipolar, horizontal, and amacrine cell bodies are localized and degeneration of the inner plexiform layer where many of the synaptic contacts of the amacrine cells occur. The considerable decrement in the neurochemical markers for the GABAergic and cholinergic neurons, including the biosynthetic enzymes for their respective neurotransmitters, their neurotransmitters themselves, and the specific high-affinity uptake processes of these neurons, suggests that these amacrine neuronal types are particularly sensitive to the toxic effects of kainic acid. This interpretation must, however, be tempered by recognition of the fact that marked morphologic changes occur in the other layers of the retina after kainate injection. It is currently unclear whether these structural alterations reflect a direct effect of kainate or are secondary to the severe loss of bipolar, horizontal, and amacrine cells in the inner nuclear layer.

The changes in the neurochemical processes involving dopaminergic neurotransmission as a response to kainic acid treatment are more complicated. The presynaptic neurochemical markers for the dopaminergic neurons, tyrosine hydroxylase and endogenous dopamine, decrease only 50% after intraocular injection of 120 nmol. of kainic acid. Injections of kainic acid up to 600 nmol. do not result in further reduction in the activity of tyrosine hydroxylase. This dose-response curve, therefore, suggests that dopaminergic neurons do not exhibit a lower sensitivity to kainic acid but rather that there is a subpopulation of dopaminergic neurons that is insensitive to its toxic effects. This interpretation is compatible with the histologic findings in our study and from previous studies with monosodium glutamate that certain cells in the inner nuclear layer are unaffected by the neuroexcitatory agents.

There is considerable biochemical and electrophysiologic evidence that the postsynaptic effects of dopamine are mediated by a dopamine-sensitive adenylate cyclase. In a microdissection study of the mouse retina, adenylate cyclase activity has been shown to be concentrated in the bipolar plus ganglion cell layer. Treatment with kainic acid did not significantly affect the activity of basal adenylate cyclase in the retina. However, in the treated retinas, there was a 90 percent loss in the activation of adenylate cyclase by dopamine. Since dopaminergic amacrine cells in the retina have been shown to make synaptic contact primarily with other amacrine cells in the inner plexiform layer, the observed 90 percent loss in the putative dopamine receptor probably reflects the degeneration of postsynaptic neurons bearing the receptor. The lack of effect of kainic acid treatment on the basal adenylate cyclase activity in contrast to the near complete loss of dopamine-sensitive cyclase suggests that these two forms of adenylate cyclase have different cellular localizations.

These studies indicate that kainic acid is an agent with potent neurotoxic effect on many cells of the chick retina and in par-
ticular on the cholinergic and GABAergic amacrine cells. With administration by direct intraocular injection, the toxic actions of the drug are limited to the retina of the injected eye, and serious complications resulting from systemic injection such as seizures can be avoided. Unlike the neurotoxicity of glutamate, which appears to be restricted to immature retina, kainic acid exerts its toxic effects in the relatively fully differentiated chick retina. In situ injection of kainic acid is an effective pharmacologic technique for lesioning interneurons that can be helpful in identifying their functional role as well as their neurochemical characteristics. The neurotoxic effects of the drug are not limited to the retina since stereotaxic injection of kainic acid into the rat striatum induces degeneration of cholinergic and GABAergic neurons intrinsic to this region of the brain, as we recently demonstrated.

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REFERENCES

