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Alterations of the Electroretinogram by Intravitreal Kainic Acid in the Rat

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Purpose: To relate the electrophysiological changes in the retina induced by the excitatory neurotoxin, kainic acid (KA), to its receptor sites in the rat.

Methods: Fifty-five Wistar rats were injected intravitreally with 100, 50, 25, 12.5, 6.25, 3.12, or 1.56 nmol of KA. The electroretinograms (ERGs) including oscillatory potentials (OPs) elicited by a series of increasing intensities were recorded before, and 6 hours, 1 day, 1 week, and 1 month after the injection of KA.

Results: After KA injection, the a-waves showed no significant change at all intensity levels ($P > .05$), but the amplitudes and implicit times of the b-wave were significantly altered. The abolition of the b-wave by KA resulted in a negative response, which decreased progressively with time. The implicit times of the b-wave showed a marked prolongation after injection of 100 nmol of KA ($P < .01$). The OPs disappeared at the KA dose of 6.25 nmol and higher; doses of 1.56 to 3.12 nmol of KA depressed the Ops.

Conclusions: We conclude that KA altered the above-mentioned ERG components in a dose-dependent manner. These alterations of the ERG can be explained by alterations of neurons in the inner retinal layers. **Jpn J Ophthalmol 1999;43:495–501**

Key Words: Electroretinography, kainic acid, rat.

Introduction

Glutamate (Glu) and its related excitatory amino acids are thought to mediate excitatory synaptic transmission at the photoreceptor/bipolar cell synapses and at the bipolar/ganglion cell synapses, acting through activation of glutamate receptors. The effects of Glu are mediated by two main classes of receptors: ionotropic and metabotropic receptor subtypes.[1] The former has been named according to their preferred agonists, the N-methyl-d-aspartate (NMDA), α-amino-3-hydroxyl-5-methyl-isoxazol-4-propionic acid (AMPA), and 2-carboxy-3-carboxymethy-4-isopropenyle-pyrrolidine (kinate, kainic acid [KA]) receptors. The subunit mRNAs of the different KA receptors in the rat retina are differentially distributed throughout the inner nuclear layer (INL) and ganglion cell layer (GCL) in the horizontal cells, bipolar cells, amacrine cells, and ganglion cells.[2] KA is a conformationally restricted analogue of the excitatory amino acid, L-glutamic acid, and has potent excitatory as well as toxic actions on neurons in many regions of the vertebrate brain: in particular, the striatum, cerebellum, hippocampus, cochlear nucleus, and retina.[3–5] An intraocular injection of KA in chicks is known to cause a rapid and selective alteration in the morphology of the INL and inner plexiform layer (IPL), while essentially sparing the photoreceptors and ganglion cells.[6] Damage to the ganglion cells by intravitreal KA has also been reported in mammalian[7–9] but not in chick retinas.[6,10–12] Thus, intravitreal KA induces a species-specific and cell-specific neuronal death in the retinas of many vertebrates.

The effects of KA on retinal morphology[6,10–14] have been widely investigated, but only a few studies have used electroretinographic (ERG) methods to study the retinal damage. Because the b-wave[15,16] and oscillatory potentials (OPs)[17,18] of the ERG re-

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flect the neural activity of the inner retinal layers, where the neurotoxic action of KA has been shown to be most effective, the ERG may be a more sensitive method to detect KA toxicity than histological methods. One important advantage of the ERG is that the evolution of the retinal damage can be followed in the same animal over a long period. We report the changes in the ERG of the rat retina after intravitreal injection of different concentrations of KA.

Materials and Methods

A total of 55 female Wistar rats 6 weeks of age and weighing 100–120 g were used. The rats were maintained on a 12-h:12-h light/dark cycle. The animals were anesthetized by an intramuscular injection of a mixture of urethane (500 mg/kg), ketamine (11 mg/kg), and xylazine (14 mg/kg), and the pupils were dilated with topical 0.5% cyclopentolate. Experiments were carried out in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Kainic acid (Sigma, St. Louis, MO, USA) was dissolved in sterile physiological saline solution and administered at doses of 100, 50, 25, 12.5, 6.25, 3.12, and 1.56 nmol in a volume of 5 μL. Seven rats were given a dose of KA; there were six rats in the control group. After anesthesia, a paracentesis of the anterior chamber was performed and the KA solution was injected into the mid-vitreous of the right or left eye with a 30-gauge needle on a Hamilton microsyringe. The control eyes received an intravitreal injection of 5 μL of 0.9% saline. The eye receiving the KA injection was selected randomly. All these procedures were carried out under an operating microscope.

The light source for stimulation was a 150-W quartz halogen light bulb. The light was collected and focused onto a 3-mm-diameter fiber optic bundle, and the other end of the fiber optic bundle was positioned approximately 5 mm above the cornea. The illuminance of the unattenuated stimulus on the surface of the cornea was 1.4×10^5 lux; neutral density filters (NDFs) were used to reduce the stimulus intensity. The ERG signals were amplified (MEG-2100; Nihon Kohden, Tokyo) and the bandpass was set at 1.5–100 Hz for the a- and b-waves, and at 150–300 Hz for the OPs. The duration of the stimulus and interstimulus interval were set at 20 milliseconds and 5 seconds, respectively, by an electromagnetic shutter. Eight responses were averaged (DS-6411; Iwatsu Electric, Tokyo). The interval from one intensity to another intensity was set at 1 minute.

Each rat was placed on its side with its head fixed in place by surgical tape, and was dark-adapted for 30 minutes. The ERGs were recorded between a silver–silver chloride cotton wick electrode placed on the cornea and a stainless steel needle electrode placed subcutaneously on the nasal bone. Another electrode was placed subcutaneously in the neck area as ground. Rats were placed in a small electrically shielded cage and the rectal temperature was kept at 38°C with a heating pad. ERGs were recorded with a fixed set of stimuli with the intensity increased in 1.0-log steps from 6.0-log NDF to the maximum intensity available.

The a-wave amplitude was measured from the baseline to the trough of the a-wave; the b-wave amplitude was measured from the trough of the a-wave to the positive peak. The implicit times of both a- and b-waves were measured from the flash onset to the trough or peak. The mean ± standard error of the mean (SEM) for the amplitudes and implicit times were calculated. Statistical significance of the difference between the control and the KA-injected eye was determined with the two-tailed Student's *t*-test.

Results

Figure 1 shows the dark-adapted ERG intensity series of a control rat before and after the saline injection. All the ERGs were recorded from the same rat and each ERG is the average of eight responses. In this control eye, the a- and b-wave amplitudes

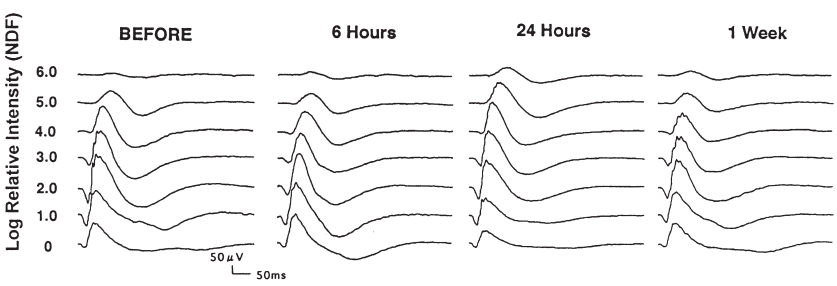

Figure 1. Electroretinograms recorded from Wistar rat before, 6, and 24 hours after, and 1 week after intraocular injection of 5 μL of 0.9% saline. Number on left represents value of neutral density filter (NDF), which was used to reduce full intensity stimulus in log units. Maximum illuminance (NDF = 0) is 1.4×10^5 lux.

were slightly reduced at 6 hours, increased at 24 hours and recovered to initial level at 1 week. Similar findings were noted in the other control eyes, and statistical analysis proved that the changes were not significant. The implicit times of the a- and b-waves, also, did not show any significant changes ($P > .05$). These findings demonstrate that the procedures, viz, the hypotonia caused by the paracentesis and the increase in the intraocular pressure caused by the intravitreal injection, did not affect the ERGs permanently.

Figure 2 shows the ERGs recorded after the injection of 100 nmol of KA. The amplitude of the b-waves decreased and the decrease was significant at 6 hours ($P < .05$) for all intensities. After longer periods, the b-wave became very small, leaving a negative-type ERG. The implicit times of the b-wave were significantly prolonged even 6 hours after the injection. This delay was maintained at 24 hours and at longer periods ($P < .01$).

The mean of the implicit times and the amplitudes of the a-wave at various periods after the KA injection are plotted as a function of KA concentration in Figures 3A and 3B. The bars represent the SEM. The results at each period after the KA injection are shown by the different curves. Both parameters of the a-wave were not significantly altered by KA.

In the same way, Figure 4 illustrates the effect of various doses of KA on the mean (±SEM) implicit times (Figure 4A) and the mean (±SEM) b-wave amplitudes of the b-waves (Figure 4B) elicited by 1.0-log NDF stimulus at different testing times. As opposed to the a-wave, a distinct dose-dependent effect was found for the b-wave. The amplitude of the b-waves at doses of 12.5 nmol and higher showed a pattern similar to that with 100 nmol/L KA (Figure 2), a rapid decrease at 6 hours and further decrease at 24 hours and 1 week. At these higher doses, the b-wave did not recover after longer periods. At the lower doses, the changes in the b-wave amplitude were more variable.

The implicit time of the b-wave recorded 6 hours after injection of 3.12 to 50 nmol of KA was significantly prolonged ($P < .05$). The implicit time then recovered at 6 hours and did not significantly differ from the pre-KA implicit time ($P > .05$) (Figure 4A).

Figures 5A to 5D show the intensity-response curves for the mean b-wave amplitude for KA doses of 100, 25, 6.25, and 1.56 nmol, respectively. In general, these doses of KA did not alter the shape of the intensity-response curves; the amplitude of the b-wave increased with increasing stimulus intensity until saturation and then decreased. The maximum b-wave amplitudes (V_{max}) were elicited with the 1.0-log NDF. With doses of 12.5 nmol and higher, the b-wave was significantly reduced after 24 hours, and a negative type ERG component was recorded. This negative wave remained throughout the test period. After injection of 6.25 nmol of KA, the b-wave recovered at 24 hours (Figures 4B, 5C). With 3.12 nmol, a recovery was noted 6 hours after injection, although the b-wave was still smaller than before injection (Figure 4B). With 1.56 nmol of KA, we did not find any significant changes in the ERG components examined in this report (Figure 5D).

In the control saline-injected eyes, the OPs were not altered. The OPs elicited with 1.0-log NDF stimulus intensity at various periods after the KA injection are shown in Figure 6. At the higher concentrations of KA (6.25 nmol and higher), the OPs were not recordable after 6 hours, and did not recover in any of the injected eyes. At the lower concentrations (3.12 nmol, 1.56 nmol), the changes were not consistent: with 3.12 nmol in 2 eyes, the OPs were significantly reduced but were recordable at 6 hours and at later times; with 1.56 nmol, in one eye OPs were abolished and other eyes showed no change in the OPs.

Discussion

The absence of a permanent change after saline injection showed that the mechanical procedures were not responsible for the changes observed with KA.

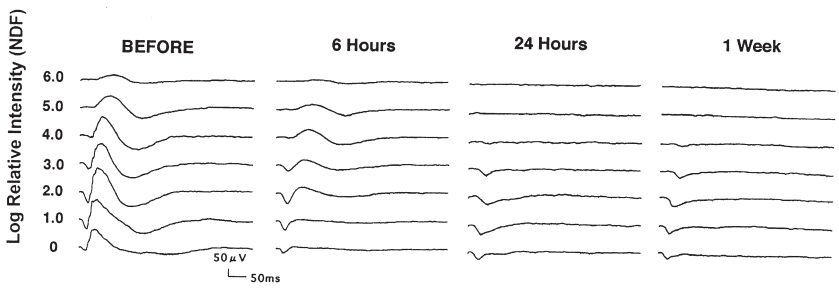

Figure 2. Electroretinograms of rat injected intravitreally with 100 nmol of kainic acid.

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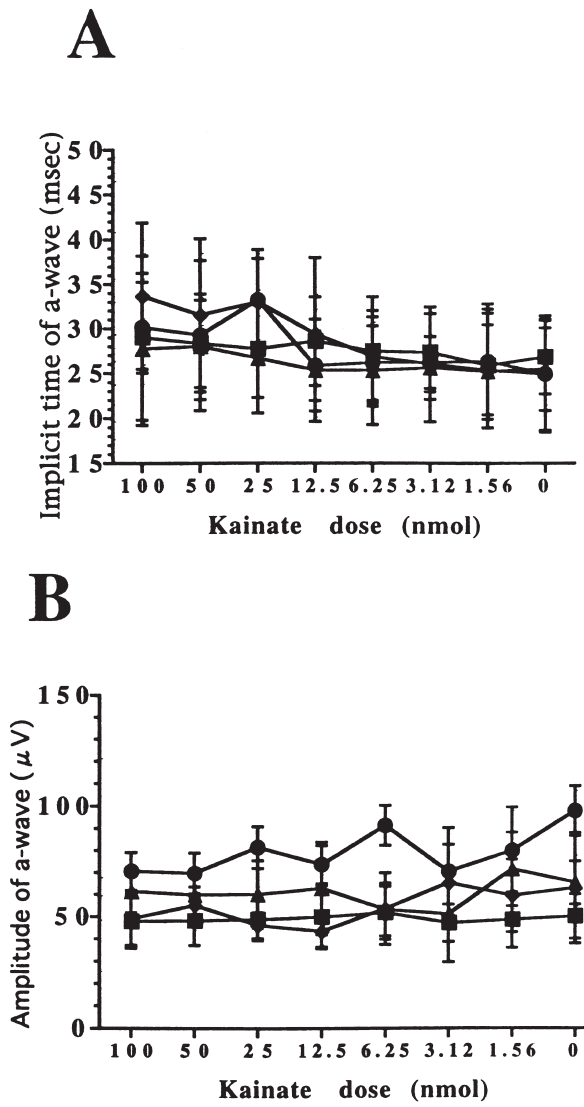

Figure 3. Plots of mean implicit times (**A**) and amplitudes (**B**) of a-wave from rats (n = 7) after intravitreal injection of various doses of kainic acid. Light intensity is 1.0-log neutral density filter. ■: before, ▲: 6 hours, ●: 24 hours, ◆: 1 week.

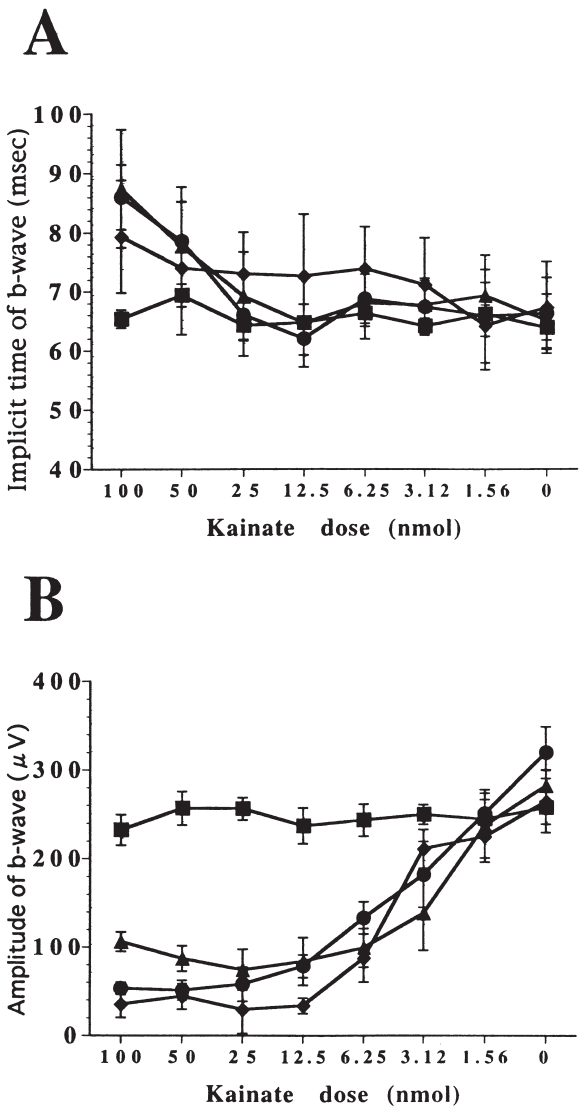

Figure 4. Effects of various doses of kainic acid (KA) on mean amplitude (**A**) of b-wave (V_{max}) and implicit time (**B**) of b-wave elicited at stimulus intensity of 1.0-log neutral density filter. Values represent mean ± SEM (n = 6 for KA 0; n = 7 for KA 1.56-100). ■: before, ▲: 6 hours, ●: 24 hours, ◆: 1 week.

The alterations in the b-wave and the OPs of the ERG showed that the rat retina was very sensitive to KA, with a threshold dose of about 3.12 nmol/L for ERG changes, which is significantly lower than the doses reported earlier for doses affecting the retina.[7,8,10–14] Vaegan and Millar[8] demonstrated that the mean b-wave amplitude-intensity function was close to normal for all doses up to and including 50 nmol, and that the b-wave V_{max} was reduced by 50% at 100 nmol in the cat. However, a simple comparison of the doses of KA is not valid because in the earlier reports, cats and chicks were used.[7,8,10–14] The intravitreal concentration difference depending on the volume of vitreous should be taken into account. Our results showed that KA reduced b-wave amplitude in nearly all doses from 3.12 nmol/L to 100 nmol/L, but only 100 nmol KA delayed the implicit time of the b-wave. This may be related to several factors, such as sensitivity differences between the neurons of different species and differences in electrophysiological recording conditions.[8] We can at least say

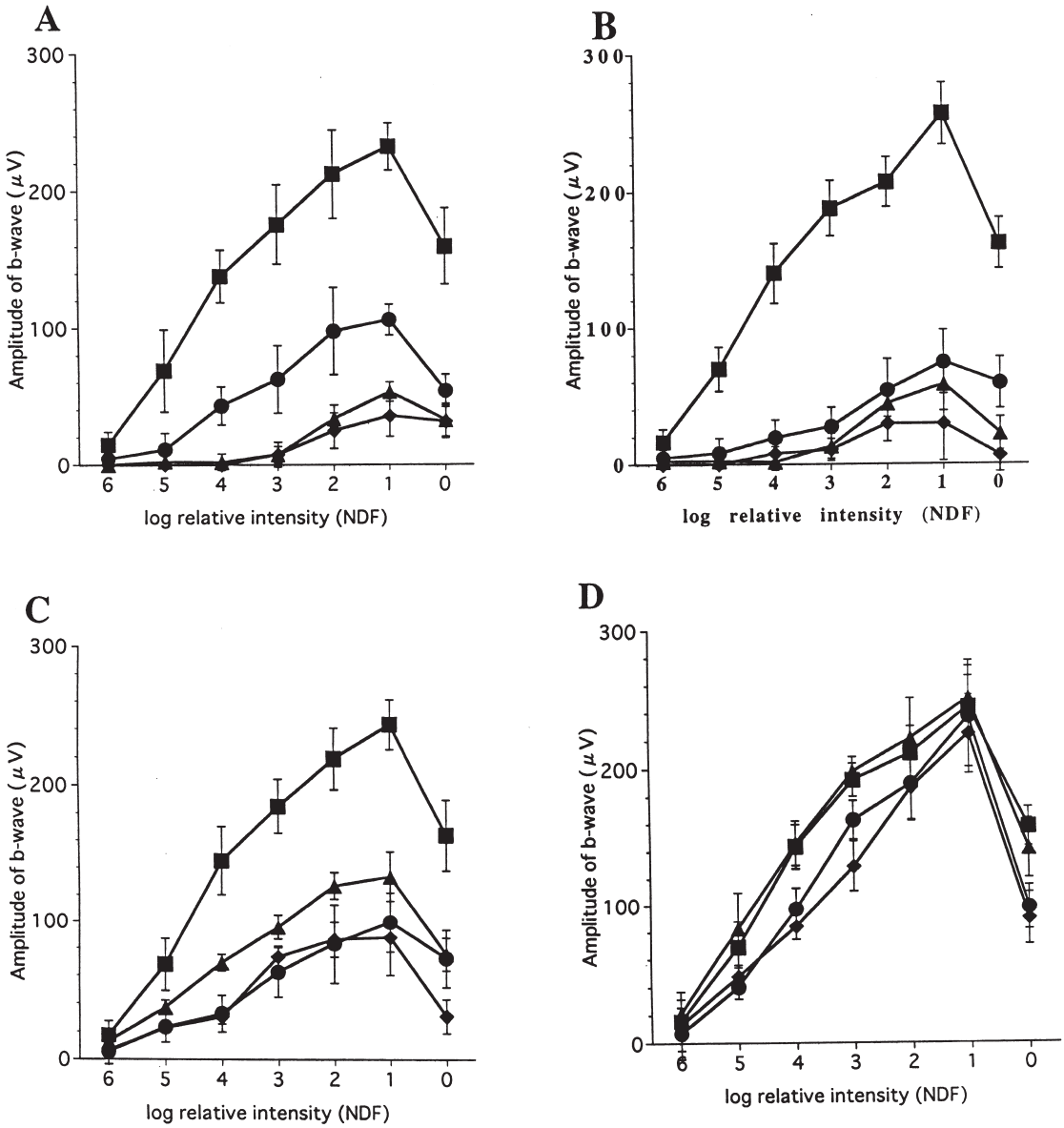

Figure 5. Amplitude of b-wave (mean ± SEM) versus log stimulus intensity before (■), 6 hours (▲), 24 hours (●), and 1 week (◆) after intravitreal injection of 100 nmol (**A**), 25 nmol/L (**B**), 6.25 nmol (**C**), and 1.56 nmol (**D**) of kainic acid (n = 7). NDF: neutral density filter.

that the implicit time of the b-wave was little affected even by a very high dose in a very small volume.

Interestingly, the effect of KA is a relatively slow process and the changes occurred over a 24-hour period (Figures 2 and 4). The implicit times of the b-wave also showed changes over a 24-hour period.

Our results are in agreement with Goto et al[9] who demonstrated decreased b-waves at 2 hours and almost complete abolition by 24 hours after injection of 50 and 200 nmol of KA in the rat. Our results, showing that the waveform at 1 month is similar to that at 1 week, are in agreement with the histological observations by Dvorak and Morgan[10] and demonstrate that the changes are permanent.

The most prominent component, the b-wave, is generally thought to be initiated by depolarization of on-bipolar cells in response to light, inducing potassium efflux from Müller cell endfeet.[15,16] The decrease of the b-wave amplitudes could have resulted from a physiological alteration of the cells, which

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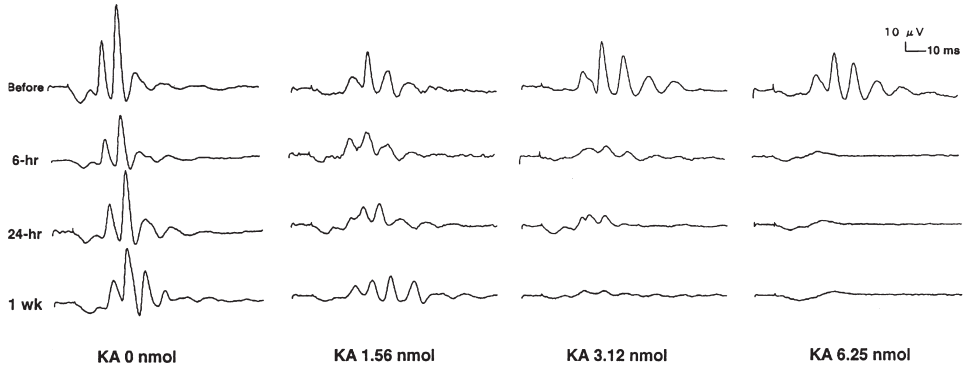

Figure 6. Oscillatory potentials (OPs) recorded before, and 6 hours, 24 hours, and 1 week after intravitreal injection of different doses of kainic acid. Oscillatory potentials were elicited at stimulus intensity of 1.0-log neutral density filter (n = 7).

give rise to the b-wave, or a reduction in the number of these cells because of death, or both. This is consistent with previous histological findings that KA selectively damages the outer plexiform layer, INL, IPL, and GCL after intravitreal injections, and the lesions are dose-dependent.[9]

Kainic acid, which is an agonist for non-NMDA glutamate receptors, is probably the most effective neurotoxin used to "selectively" damage the retina.[3–6,9] It is generally thought that KA kills cells by overstimulating kainate/AMPA receptors. This is supported by the fact that the KA effect can be attenuated by administration of kainate/AMPA receptor antagonists.[1,19,20] Because an intraocular injection of KA causes the death of various neurons (bipolar, amacrine, and ganglion cells), an alteration of the b-wave is to be expected. When 12.5 nmol or more KA is injected, the responses consist of negative-type ERGs after 24 hours.

The OPs represent neuronal activities in the inner part of the retina and reflect rapid adaptational changes.[17,18] OPs have been reported to be affected by excitatory amino acids[18] and to be the electrophysiological component most sensitive to KA.[7,8] Our results in rats in this study are consistent with this conclusion. Another Glu analogue, 2-amino-4-phosphonobutyric acid, selectively abolished the b-wave while preserving OPs.[21] The present results, together with previous reports,[7,8,21] suggest the close correlation between the putative site of origin of the OPs and the neurons most sensitive to KA toxicity. We found that the OPs were the first component affected by KA, and that even at the lowest concentration of 1.56 nmol/L, the OPs were rapidly reduced but then gradually recovered along with b-waves. With a higher dose of 6.25 nmol, KA eliminated the OPs permanently.

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