大韓獸醫學會誌 (1986) 第26卷 第2號
Korean J. Vet. Res. (1986) Vol. 26, No. 2, pp. 221~224

Effects of Kainic acid on the Duck Retina

Tae-kyun Shin and Moo-gang Kim

Department of Anatomy, College of Medicine, Chungnam National University
(Received July 30th, 1986)

Kainic acid가 오리 망막에 미치는 영향

신 태 균·김 무 강

충남대학교 의과대학 해부학교실
(1986. 7. 30 接受)

초 록 : Kainic acid가 오리 망막에 미치는 영향을 알아보기 위하여 kainic acid 50μl(1.0μmol)를 오리의 안구내로 주입한 후 망막을 경시적으로 관찰하였던 바 다음과 같은 결과를 얻었다.

주사후 8시간째 망막의 내내핵층과 내망상층에서 공포화가 인정되었고 24시간째는 내핵층에서 핵농축이 현저하였으며 48시간째의 예에서는 농축된 핵이 상당수 소실되면서 망막의 두께도 다소 얇게 나타났다. 주사후 96시간과 10일째는 내핵층을 구성하는 대부분의 세포가 소실되어 망막의 두께가 상당히 얇아졌고 내망상층의 두께도 경시적으로 얇아지는 경향이었다.

Introduction

Systemic administrations of monosodium glutamate to immature animals cause the degeneration of certain neurons of retina(Olney, 1968) and brain(Olney *et al.*, 1972; Burde *et al.*, 1971; Olney, 1969). A heterocyclic rigid analogue of glutamate, kainic acid, has been shown to be considerably more potent as a neuroexcitant than glutamate itself(Johnston *et al.*, 1974). Schwarcz and Coyle(1977) have reported that intraocular injections of kainic acid destroy amacrine cells in the inner nuclear layer of the chick retina.

To provide further informations on the kainic acid-induced retinal lesions in the avian species, the histologic characteristics of the duck retina intraocularly injected with kainic acid were examined.

Materials and Methods

Treatment with kainic acid: Ten young domestic ducks(Anas platyrhyncha), 7-day-old, were obtained from a market. Kainic acid(Sigma Chemical Co., St. Louis, Mo.) was dissolved in sterile normal saline solution and titrated to pH 7.4; 1μmol. of the kainate in a volume of 50μl was injected into right eyes intraocularly. Left eyes received intraocular injection of saline as controls. Two ducks were killed by decapitation at various times such as 8, 24, 48, 96 hours and 10 days after injection.

Preparation of tissues: After decapitation the eyes were removed and fixed in 2% glutaraldehyde+2.5% paraformaldehyde in 0.05M cacodylate buffer(pH 7.4) for 2 hours at 4°C, washed in 0.1M cacodylate buffer (pH 7.4) for 1 hour, and post-fixed in 1% osmium tetroxide in 0.1M cacodylate buffer(pH 7.4) for 1 hour at 4°C. Materials were then dehydrated in a graded series of ethanol, passed through propylene oxide at room temperature and embedded in Epon 812. Semi-thin sections were cut on an ultramicrotome

(Sorvall MT-5, 000) and stained with 1% methylene blue for light microscopy.

Results

All control eyes showed well preserved retinas.

By 8 hours after injection the inner plexiform layer had a "cheese" appearance with large vacuoles. There were vacuolar degenerations in the cells of the inner nuclear layer, these changes were particulary severe in the inner portion of the inner nuclear layer. The outer layers of the retina were intact (Fig. 1).

By 24 hours after injection many of the cells exhibited pyknotic nuclei in the inner nuclear layer, the reduction in the thickness of this layer was apparent. There were also some pyknotic nuclei in the ganglion cell layer (Fig. 2).

By 48 hours after injection there were some pyknotic nuclei and the loss of cells in the inner nuclear layer, the number of pyknotic nuclei was more or less small compared to the cases at 24 hours after injection. Vacuolar degenerations were also recognized in the outer plexiform layer although the outer nuclear layer was intact (Fig. 3).

By 96 hours after injection there were only a few cells in the center and inner portion of the inner nuclear layer except for the cells located in the outer zone of this layer. Whereas the pyknotic nuclei were seen no more in this time. The number of ganglion cells appeared to be slightly reduced, the glia cells were intact in the ganglion cell layer.

By 10 days after injection there was a decrease in the thickness of the inner nuclear layer and inner plexiform layer; the contraction of the inner nuclear layer was due to a marked reduction in the cell number, the contraction of the inner plexiform layer was secondarily due to the loss of the amacrine and the bipolar cells in the inner nuclear layer, which the axons of the amacrine and the bipolar cells made up the inner plexiform layer. Consequently the number of ganglion cells reduced. There were also severe vacuolar degeneration in the outer plexiform layer (Fig. 4).

Discussion

Monosodium glutamate destroys neurons of the inner retina or arcuate nucleus of the hypothalamus when administered subcutaneous to infant animals of several species(Olney *et al.*, 1972; Burde *et al.*, 1971; Olney, 1969). Kainic acid, a heterocyclic rigid analogue of glutamate, has been shown to be considerably more potent as a neuroexicitant than glutamate itself(Johnston *et al.*, 1974; Olney *et al.*, 1974). Since the systemic administration of kainic acid exerts neurotoxic effects on central nervous system neurons, its marked toxicity resulting in seisures and death has limited its experimental use (Olney *et al.*, 1974). Stereotaxic injection of kainic acid into the rat striatum induced degeneration of cholinergic and GABAergic neurons intrinsic to this region of the brain(Coyle and Schwarcz, 1976).

In the present study by 8 hours after injection, vaculation of the cells in the inner portion of the inner nuclear layer, where the amacrine cells predominate, suggests that these amacrine neuronal types are particularly sensitive to the toxic effects of kainic acid. There was also pyknosis in the inner portion, and in the center of the inner nuclear layer by 24 hours and 48 hours after injection, progressively loss of cells in this layer as time lapses by 96 hours and 10 days after injection.

There was degeneration of the inner plexiform layer where many of the synaptic contacts of the amacrine and bipolar cells occur. These results reported here were similar to the cases of kainic acid-retinal toxicity in chick(Schwarcz and Colye, 1977), however Schwarcz and Coyle(1977) did not show the progressive changes of retina induced by kainic acid.

Gibson *et al.* (1985) hypothesize that Quinolinate receptors may be confined to the inner plexiform layer, Biziere and Coyle(1979) suppose that kainic acid receptors seem like to be present in both inner plexiform and outer plexiform layer. The present study here also supports that kainic acid receptors seem to be present particularly in the inner plexiform layer in relation to the inner portion of inner nuclear layer and also to be in the outer plexiform layer although the vacuolar degenerations are not severe.

This study indicates that kainic acid is an agent with potent neurotoxic effect on many cells of the duck retinas and particularly on the amacrine cells

and injection of kainic acid is an effective agent for lesioning interneurons that can be helpful in identifying their functional role.

Conclusions

Authors evaluated retinal toxicity produced by intraocular injection of kainic acid in 10 ducks to provide further informations on the kainic acid-induced retinal lesions in the avian species.

By 8 hours after injection there were severe vacuolar degenerations in the inner plexiform layer and the inner portion of the inner nuclear layer; by 24 hours after injection many of the cells exhibited pyknotic nuclei in the inner nuclear layer; by 48 hours after injection the reduction in the thickness was apparent in this layer because of the partial loss of the pyknotic cells. By 96 hours there were only a few cells in the center and inner portion of the inner nuclear layer; by 10 days there was a conspicuous decrease in the thickness of the inner nuclear layer and the inner plexiform layer.

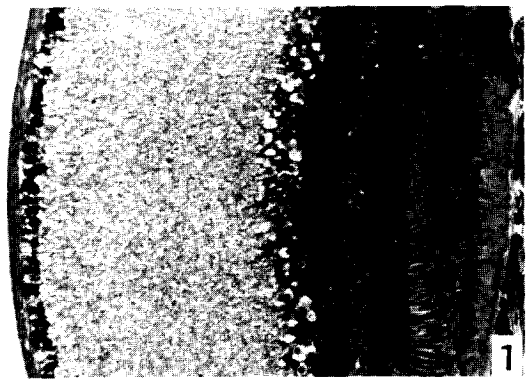
Legends for Figures

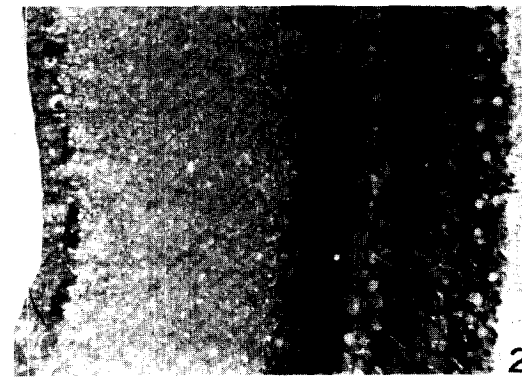
Fig. 1. Vertical section of the duck retina by 8 hours after intraocular injection of kainic acid. There are severe vacuolar degenerations of the cells in the inner portion of the inner nuclear layer. Methylene blue. ×80.

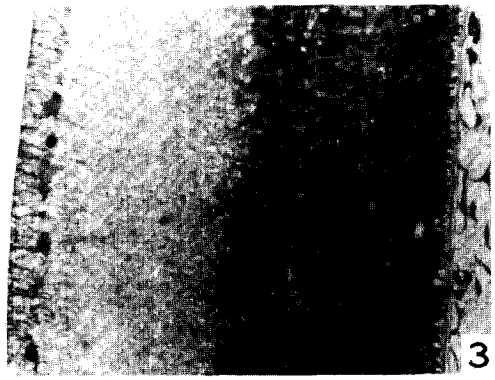
Fig. 2. Duck retina by 24 hours. Many pyknotic nuclei occur in the inner nuclear layer and one cell shows pyknotic nucleus in the ganglion cell layer. Methylene blue. ×112.

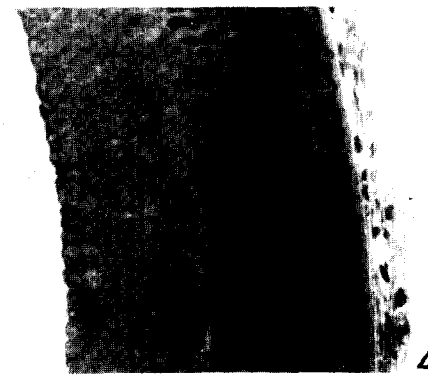
Fig. 3. Duck retina by 48 hours. The number of pyknotic nuclei are more or less small compared to the Fig. 2. Methylene blue. ×112.

Fig. 4. Duck retina by 10 days. There is a decrease in the thickness of the inner nuclear layer and inner plexiform layer; the loss of cells in the inner nuclear layer is nearly complete. Methylene blue. ×112.









References

Biziere, K. and Coyle, J.T. (1979) Localization of receptors for kainic acid in neurons in the inner nuclear layer of retina. Neuropharmacology, 18:409~411.

Burde, R.M., Schainker, B. and Kayes, J. (1971) Monosodium glutamate: Acute effect of oral and sucutaneous administration on the arcuate nucleus of the hypothalamus in mice and rats. Nature, 233:58~60.

Coyle, J.T. and Schwarcz, R. (1976) Model for Huntington's chorea: Lesion of striatal neurons with kainic acid. Nature, 263:244.

Gibson, B.L., Schwarcz, R. and Reif-Lohrer, L. (1985) Quinolinate: A selective neurotoxin in embryonic and posthatching chicken retinas. Invest. Ophthalmol. Vis. Sci., 26:50~57.

Johnston, G.A.R., Curtis, D.R., Davies, J. and McCulloch, R.M. (1974) Excitation of spinal interneurons by some conformationally restricted analogues of L-glutamic acid. Nature, 248:804.

Olney, J.W. (1968) Glutamate-induced retinal degeneration in neonatal mice: Electron microscopy of acutely evolving lesion. J. Neuropathol. Exp. Neurol., 28:455~460.

Olney, J.W. (1969) Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. Science, 164:719~721.

Olney, J.W., Sharpe, L.G. and Feigin, R.D. (1972) Glutamate-induced brain damage in infant primates. J. Neuropathol. Exp. Neurol., 31: 464~488.

Olney, J.W., Rhee, V. and Ho, O.L. (1974) Kainic acid: A powerful neurotoxic analogue of glutamate. Brain Res., 77:507~512.

Schwarcz, R. and Coyle, J.T. (1977) Kainic acid: Neurotoxic effects after intraocular injection. Invest. Ophthalmol. Vis. Sci., 16:141~148.