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KAINIC ACID DEPLETES RETINAL PHENYLETHANOLAMINE-N-METHYLTRANSFERASE ACTIVITY OF RATS

JOSEPH COHEN

Department of Pharmacology, Graduate School of Arts and Sciences and College of Medicine, Preclinical Building, Room 3412, 520 W Street., N.W., Washington, D.C. 20059

ABSTRACT. Kainic acid, an excitotoxic agent to the retina as well as to neuronal cell bodies in the brain, was administered intraocularly to rats in order to study the sensitivity of phenylethanolamine-N-Methyltransferase (PNMT) containing amacrine cells to this agent. Results show that these cells are very sensitive to the toxic effects of kainate. A dose of 5 nmoles caused a significant reduction in retinal PNMT activity. Higher doses further depleted enzyme activity.

INTRODUCTION. The enzyme PNMT, along with its synthetic product epinephrine, is present in vertebrate retina (1). Immunohistochemical studies of the rat retina have located PNMT immunoreactivity to a subpopulation of amacrine cells located in the inner nuclear layer. These cells project their terminals to the inner plexiform layer (2). Additional studies have shown that the majority of these cells are PNMT-specific and do not contain other enzymes involved in the biosynthetic pathway of catecholamines (3,4). Cells of the retina, like neuronal cell bodies in the brain, are sensitive to the toxic effects of various excitant amino acids (5). Kainic acid, a rigid analog of glutamate, is the most potent of these agents (6).

Cells located in the inner nuclear layer are particularly sensitive to its toxic effects (5,7). These include amacrine cells that contain dopamine (8), acetylcholine (9), and various peptides (7,9). Still, instances have been found where both kainic acid sensitive and resistant populations of amacrine cells may exist among specific subtypes of cells. The susceptibility of PNMT-containing amacrine cells to kainic acid has not been examined. This investigation was designed to examined the vulnerability of such cells to the toxic effects of this agent.

METHODS. Various doses of neutralized kainic acid solution were injected in 5 ul volume into the vitreous of right eyes and saline into the vitreous of left eyes of adult male Sprague-Dawley rats, 175-200 grams. Animals were under ether anesthesia during the injection. Animals were sacrificed at various times after injections, eyes enucleated and retinas removed and frozen at -80°C and assayed for enzyme activity within one week.

PNMT activity was measured by the method of Axelrod (10) as modified by Moore and Phillipson (11). A single retina was sonicated in 75 ul of 0.005 M Tris-HCl buffer, pH 8.6, containing 0.2% Triton-X-100. The homogenate was centrifuged at 2°C for 25 minutes at 30,000 x g and the supernatant used for the enzyme assay. To centrifuge tubes

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containing 65 ul of 0.25 M Tris-HCl buffer pH 8.6 were added 25 ul of supernatant, pargyline to a final concentration 63 uM, d,l-phenylethanolamine to a final concentration of 0.35 mM, and [^{3}H] S-adenosine-L-methionine (2 Ci/mmol, New England Nuclear, Boston, MA) to a final concentration of 1.1 uM. Final volume of the assay tube was 105 ul. Tubes were incubated at 37°C in a shaking water bath, 40 oscillations per minute. The reaction was terminated at the end of 30 minutes by the addition of 200 ul of 0.5 M borate buffer, pH 10, followed by vigorous mixing for a few seconds. Three ml of toluene: isoamyl mixture, 97:3, were added to tubes followed by vigorous mixing. Tubes were centrifuged for 5 minutes to separate the organic and aqueous phases. The organic phase, containing the methylated product N-methylphenylethanolamine, was removed and placed in scintillation vials and allowed to evaporate to dryness. Radioactivity in the vials was determined by adding 10 ml of Econofluor containing 10% methanol followed by counting in a liquid scintillation spectrometer. Protein was measured by the procedure of Lowry et. al (12).

Statistical anaylsis was performed by Student's t-test for paired samples.

RESULTS. Five, 10 or 50 nmol of kainic acid was injected into the right and saline into the left eyes. At the end of one week, PNMT activity was measured as stated above. Enzyme activities of control eyes from each group of rats were similar and were not statistically different. Activities of control eyes were 6.6 $\pm$ 0.21, 7.4 $\pm$ 0.31, and 6.8 $\pm$ .24 from animals whose opposite eyes were injected with 5, 10, or 50 nmol of kainic acid respectively. Five, 10 and 50 nmol of kainic acid significantly reduced PNMT activity ($p < 0.0005$) to 62, 17 and 6.5% of control activity respectively (Figure 1).

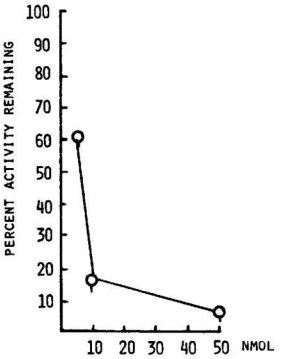

Figure 1. Depletion of Retinal PNMT Activity of Rats by Different Doses of Kainic Acid.
Different doses of kainic acid were administered intravitreally to the right eyes and saline to the left eyes of rats. Animals were sacrificed one week later. Retinal PNMT activities of kainic acid-treated eyes were compared to those of saline treated eyes. Activities of controls retinas ranged from 6.6 ± 0.21 to 7.4 ± 0.31 pmol prod/30 min/mg prot.

Additionally, a time response curve was constructed to the 10 nmol dose of kainic acid. This dose was chosen rather than the 50 nmol dose since retinas from eyes that received the 50 nmol injection appeared somewhat fragile. Significant depletion of PNMT activity occurred at 48 hours and near maximal depletion occurred at 96 hours (Figure 2).

DISCUSSION. A biochemical assay has been used to determine the sensitivity of PNMT-containing cells of the rat retina to kainic acid. These cells are among a subclass of amacrine cells located in the inner nuclear layer.

Histological examinations of retinas from eyes of the rat, as with other species injected with kainic acid, has well established the fact that kainic acid is especially toxic to the inner nuclear layer, while most of the photoreceptors and ganglion cells are spared. The general appearance of the retina in photomicrographs of kainic

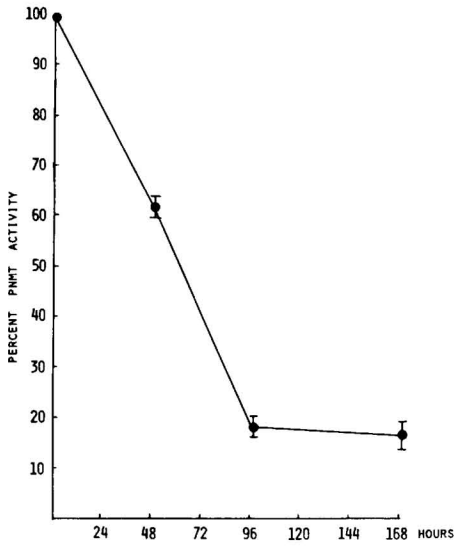

Figure 2. Depletion of Retinal PNMT Activities of Rats as a Function of Time. A 10 nmol dose of kainic acid was administered intravitreally to the right and saline to the left eyes. Animals were sacrificed at different times after injection. PNMT activities of retinas from control eyes were similar to those in figure 1.

acid-treated eyes is one of overall decreased thickness, due mostly to a reduction in cell number in the inner nuclear layer (5,7,13). Using various biochemical and immunohistochemical procedures, the identity of cell types in the inner nuclear layer that are sensitive to kainic acid can be identified. Results of this investigation show that the PNMT-containing cells are sensitive to the toxic effects of kainic acid.

While cells of the inner nuclear layer are particularly vulnerable to the toxic effects of kainic acid, cases have been reported of observations in which certain subclasses of amacrine cells possess populations of both kainic acid sensitive and kainic acid resistant types of cells. This appears to be the case in the adult rabbit retina, where increasing doses of kainic acid failed to deplete greater than 50% of somatostatin immunoreactivity, indicating that 50% of the somatostatin containing amacrine cells are resistant to the toxic effects of kainic acid (8). A similar observation has been made with the dopamine-containing amacrine cells in the chick retina (5). In this study the dose response curve indicates that with respect to kainic acid, there is only one population of PNMT-containing cells with respect to kainic acid sensitivity.

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