Alterations in Retinal Neurotransmitter Receptors and Neuropeptides of the Chick by Kainic Acid and Acrylamide

SYED FATEHYAB ALI, JAU-SHYONG HONG and STEPHEN C. BONDY*

Laboratory of Behavioral and Neurological Toxicology, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, NC 27709 (U.S.A.)

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The effects of intraocular injection of kainic acid and acrylamide upon retinal neuropeptides and high affinity binding sites have been determined in the chick. Kainic acid causes a sharp reduction in Met-enkephalin and somatostatin while neurotensin levels are unchanged. This treatment also lowers the extent of cholinergic muscarinic but not of [3H]naloxone or [3H]spiroperidol binding. In contrast, acrylamide treatment causes major increases of retinal Met-enkephalin and neurotensin concentrations. The binding of [3H]naloxone is also increased, and no reductions of any peptide or binding intensity were observed. The results indicate the plasticity of retinal neuropeptide levels and the selectivity with which these can be modulated.

INTRODUCTION

Kainic acid, a conformationally restricted analog of glutamate is an agonist of the transmitter action of glutamate. As a neuroexcitatory compound kainic acid is many times more potent than the natural amino acid and is neurotoxic when injected into nerve tissue. The cause of neuronal death by kainate is not clear; however it may be related to excessive cell depolarization caused at least in part by interaction with glutamic acid receptors. Intraocular injection of kainate into the eye of the chick produces substantial lesions of the inner nuclear and plexiform layers. Since axons terminating in or passing through a kainate treated region are predominantly uninjured while intrinsic neurons are destroyed, this toxic agent can be useful as a tool for localizing neurochemical constituents of specific cells. We have used kainic acid and another chemical known to damage nerve tissue, acrylamide, to study alterations in the retinal content of neuropeptides in the chick. The opiate binding site has also been assayed. The avian nervous system is known to exhibit measurable amounts of peptides such as Met-enkephalin, somatostatin and substance-P at a very early developmental stage and the avian retina is a rich source of neuropeptides. Since acrylamide treatment modulates the dopaminergic system of the rat striatum in a relatively specific manner, the effect of this compound upon retinal dopamine receptors was also examined.

MATERIALS AND METHODS

Chicks of a White Leghorn strain were used, and at 3 days of age were monocularly injected with 10 µl of an aqueous solution containing either 27 µg, (128 nmol) kainic acid or 50 µg acrylamide. This dose of kainate has been reported to selectively destroy amacrine and horizontal cells in the retina over a period of several days, while photoreceptors, ganglion cells, and optic fibers are relatively unaffected. The amount of acrylamide injected corresponds to a systemic dose of 50 mg/kg which causes some changes in dopaminergic circuitry in the rat brain. These changes occur within 24 h and are reversible. The eye of the chick contralateral to the treated eye was injected with 10 µl H2O and was considered to be a control. Twenty-four hours after acrylamide or 7
days after kainate injection chicks were killed by decapitation and the retina dissected out and maintained at $-70\,\degree C$.

The retinal content of Met-enkephalin (ME), substance-P (SP) and somatostatin (SRIF) was determined by radioimmunoassay. Tissue was homogenized in 2 N acetic acid then boiled for 5 min and centrifuged at 25,000 $g$ for 20 min. The supernatant was lyophilized and the residue was reconstituted with H$_2$O and radioimmunoassayed using [tyrosyl-3,5-3H]neurotensin (61 Ci/mmol), [tyrosyl-3,5-3H]Met-enkephalin (36 Ci/mmol), [125I]substance-P, [125I]somatostatin (original specific activity of iodine was 65 $\mu$Ci/$\mu$g). Antisera were raised in rabbits using polylysine conjugates. Non-labeled peptide or brain extract was incubated with antiserum and isotopically labeled peptides in containing in 2N acetic acid then boiled for 5 min and centrifuged and the residue was reconstituted with H$_2$O. The validation and specificity of this method have been described in detail.$^{10-12}$.

For the binding assay, a crude membrane fraction was prepared from brain regions by homogenization of tissue in 19 vols. of 0.2 M Tris buffer, pH 7.4, containing 0.1% albumin and 0.06% dextran. The incubation was carried out at 4 $\degree C$ for 15-24 h. The labeled peptide bound to antibody was separated from the unbound peptide by adding 0.2 ml of slurry containing 1.5% charcoal and 0.15% dextran (suspended in 0.2 M Tris buffer, pH 7.4), then aliquots of supernatant fluid were counted in a liquid scintillation spectrometer. The validation and specificity of this method have been described in detail.$^{10-12}$.

Binding incubations were carried out in triplicate in 1 ml of medium containing 40 mM Tris-HCl pH 7.4 and $10^{-9}$ [1-phenyl-4-3H]spiroperidol (23 Ci/mmol). The amount of tissue used per tube corresponded to 5-10 mg original wet weight and contained 300-400 $\mu$g protein as determined by the method of Lowry et al.$^{18}$. At the end of 15 min incubation at 37 $\degree C$ samples were filtered on glass fiber discs (25 mm diameter, 0.3 $\mu$m pore size, Gelman, Ann Arbor, MI) and washed twice rapidly with 5 ml Tris buffer.$^5$. Filter discs were then dried and counted in 5 ml of a scintillation counter at an efficiency of 38-43%. Control incubations were carried out in the presence of $10^{-6}$ M haloperidol in order to determine the extent of non-specific binding. In a parallel manner, muscarinic cholinergic receptors were assayed using $10^{-9}$ M D1-[benzilic 4,4-3H]quinuclidinyl benzilate (QNB, 29 Ci/mmol) and $10^{-6}$ M atropine as the unlabeled competing compound. The opiate binding site was measured using $10^{-9}$ M [N-allyl-2,3-3H]naloxone (50 Ci/mmol) with $10^{-6}$ M levorphanol as a competitor. In the case of this latter assay, the incubation temperature was 25 $\degree C$ and this was preceded by a preincubation (30 min, 37 $\degree C$) in the absence of isotope.$^{25}$ We felt it necessary to establish basic binding characteristics of the ligand used prior to this study. These included delineation of saturability, specificity, reversibility, and regional distribution.$^6$.

RESULTS

Seven days after injection of kainate into the chick eye, retinal content of ME, SP, and somatostatin were significantly decreased (Table I). Neurotensin levels were unchanged. The most dramatic decline occurred in the case of ME where almost 90% of the peptide disappeared. Rapid damage to chick retinal GABA and cholinergic neurons has also been reported after kainate treatment.$^{15}$.

Intraocular injection of acrylamide resulted in a dramatic increase in retinal ME content (over 3-fold) while neurotensin levels were also significantly el-

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Control content (ng/10 mg wet tissue)</th>
<th>Experimental content (ng/10 mg wet tissue)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kainate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Met-enkephalin</td>
<td>1.38 ± 0.14</td>
<td>0.16 ± 0.04*</td>
<td>-89</td>
</tr>
<tr>
<td>Substance P</td>
<td>0.52 ± 0.04</td>
<td>0.38 ± 0.02*</td>
<td>-27</td>
</tr>
<tr>
<td>Neurotensin</td>
<td>0.32 ± 0.04</td>
<td>0.33 ± 0.10</td>
<td>+5</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>3.91 ± 0.39*</td>
<td>1.78 ± 0.11*</td>
<td>-55</td>
</tr>
<tr>
<td><strong>Acrylamide</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Met-enkephalin</td>
<td>1.06 ± 0.20</td>
<td>3.46 ± 0.88*</td>
<td>+226</td>
</tr>
<tr>
<td>Substance P</td>
<td>0.56 ± 0.04</td>
<td>0.61 ± 0.06</td>
<td>+8</td>
</tr>
<tr>
<td>Neurotensin</td>
<td>0.35 ± 0.04</td>
<td>0.54 ± 0.08*</td>
<td>+54</td>
</tr>
</tbody>
</table>

* $P < 0.05$ that experimental differs from control value (Student’s two-tailed t-test).
evated (Table I). The effects of acrylamide and kainate were thus pronounced but tended to be in opposite directions. Since all the neuropeptides studied here have been reported to have a predominantly amacrine location within the avian retina, it is surprising that neurotensin levels remain unchanged. Such varying sensitivity has been suggested for CNS neurons. Alternatively a significant proportion of retinal neuropeptides may be within neurons projecting to the retina from the isthmo-optic nucleus. Acrylamide produces degeneration of the optic tract an effect perhaps mediated by the impairment of axoplasmic transport, reported in the chicken by Souyri et al. Such distal effects may be direct or consequent to abnormal protein synthesis in the nerve cell body. The elevated levels of Met-enkephalin observed in acrylamide-treated chicks may be another reflection of a non-lethal derangement of nerve tissue, perhaps related to altered activity of monoamine-containing neurons.

There has been discussion as to whether the neurotoxic effects of acrylamide are mediated by the formation of acrylamide metabolites by the liver. The changes reported here are unlikely to involve hepatic breakdown of acrylamide since the dose given, if considered equally distributed throughout the body, was very low (less than 1 mg/kg).

A rise of striatal Met-enkephalin levels takes place after chronic blockade of the dopaminergic system with haloperidol. For these reasons an attempt was made to correlate the elevated retinal Met-enkephalin concentrations following acrylamide treatment with altered dopamine circuitry. However the extent of [3H]spiroperidol binding within retinal membranes was not significantly altered by acrylamide treatment (Table II). This is in contrast to the reported increase of striatal spiroperidol binding in acrylamide-treated rats after 24 h exposure, and suggests a lack of independence of dopaminergic and enkephalin-related circuitry. The retinal dopamine receptor closely resembles its counterpart in the brain, and dopamine is the principal catecholamine of retina. However, spiroperidol is also known to bind to serotonergic receptors. The role of the enkephalin system in the retina is not known but it has been suggested to participate in light-induced neuroendocrine regulation by way of retino-hypothalamic projections.

The increased retinal Met-enkephalin found after acrylamide treatment was concomitant with a parallel increase in the binding of [3H]naloxone (Table II). However, the decreased level of this peptide seen in kainate-treated retina did not coincide with a changed level of [3H]naloxone binding. Thus, in neither case did the intensity of receptor binding show a reciprocal relationship with the concentration of enkephalin. A simultaneous rise in enkephalin levels and the intensity of opiate receptor binding sites, is however, compatible with the concept of reduced enkephalinergic activity due to blockade of release. The opiate receptor sites are not destroyed when enkephalin levels are dramatically reduced and thus are probably located in a separate cell population.

Acrylamide had no detectable effect on retinal muscarinic receptors while kainate treatment caused a significant loss of overall [3H]QNB binding (Table II). A preliminary report of a similar change in kainate-treated rat retina has appeared. Since insufficient tissue was available for a rigorous Scatchard plot, changes observed could be due to altered receptor density or affinity. Neither compound used had an appreciable effect on levels of retinal membrane proteins.

These data indicate the selective nature of chemically-induced retinal lesions. Such specificity in an increasingly well delineated neuronal population, makes the retina a useful tissue in which to study the effects of toxic agents on various neuronal classes.

**TABLE II**

<table>
<thead>
<tr>
<th>Labeled ligand</th>
<th>Control</th>
<th>Acrylamide</th>
<th>Kainate</th>
</tr>
</thead>
<tbody>
<tr>
<td>QNB</td>
<td>59 ± 2</td>
<td>61 ± 11</td>
<td>37 ± 7*</td>
</tr>
<tr>
<td>Naloxone</td>
<td>7.7 ± 0.6</td>
<td>19.5 ± 1.2*</td>
<td>8.6 ± 1.3</td>
</tr>
<tr>
<td>Spiroperidol</td>
<td>1085 ± 143</td>
<td>1113 ± 75</td>
<td>1199 ± 69</td>
</tr>
</tbody>
</table>

*P < 0.05 that results differ from control value (Fisher's least significant difference test).
REFERENCES


3 Biziere, K. and Coyle, J. T., Localization of receptors for kainic acid on neurons in the inner nuclear layer of retina, *Neuropharmacology*, 18 (1979) 409-413.


