UC Irvine
UC Irvine Previously Published Works

Title
Alterations in retinal neuropeptides of the chick by kainic acid and acrylamide

Permalink
https://escholarship.org/uc/item/0k50890v

Journal
Brain Res, 274

Authors
Bondy, SC
Ali, SF
Hong, JS

Publication Date
1983

Copyright Information
This work is made available under the terms of a Creative Commons Attribution License, availalbe at https://creativecommons.org/licenses/by/4.0/

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Brain Research, 274 (1983) 115–118
Elsevier

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.)

(Accepted January 11th, 1983)

Key words: retina — neuropeptides — receptors — neurotransmitters — kainic acid — acrylamide

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 [^{3}H]naloxone or [^{3}H]spiroperidol binding. In contrast, acrylamide treatment causes major increases of retinal Met-enkephalin and neurotensin concentrations. The binding of [^{3}H]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[21]. 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[21]. Intraocular injection of kainate into the eye of the chick produces substantial lesions of the inner nuclear and plexiform layers[22,29]. Since axons terminating in or passing through a kainate treated region are predominantly uninjured while intrinsic neurons are destroyed[29], 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[17] and the avian retina is a rich source of neuropeptides[32]. Since acrylamide treatment modulates the dopaminergic system of the rat striatum in a relatively specific manner[1] 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[15,22,23], while photoreceptors, ganglion cells, and optic fibers are relatively unaffected[3]. 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[1]. The eye of the chick contralateral to the treated eye was injected with 10 μl H_2O and was considered to be a control. Twenty-four hours after acrylamide or 7

* To whom correspondence should be addressed.

0006-8993/83/$03.00 © 1983 Elsevier Science Publishers B.V.

days after kainate injection chicks were killed by decapitation and the retina dissected out and maintained at —70 °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_2O and radioimmunoassayed using [tyrosyl-3,5-^{3}H]neurotensin (61 Ci/mmol), [tyrosyl-3,5-^{3}H]Met-enkephalin (36 Ci/mmol), [^{125}I]substance-P, [^{125}I]somatostatin (original specific activity of iodine was 65 μ Ci/μg). Antisera were raised in rabbits using polylysine conjugates. Non-labeled peptide or brain extract was incubated with antiserum and isotopically labeled peptides in 0.5 ml of 0.2 M Tris buffer, pH 7.4, containing 0.1% albumin and 0.06% dextran. The incubation was carried out at 4 °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].

For the binding assay, a crude membrane fraction was prepared from brain regions by homogenization of tissue in 19 vols. of 0.32 M sucrose followed by centrifugation (50,000 *g* 10 min). The precipitate from this step was then homogenized in distilled water pH 7.4 and recentrifuged. The final pellet was suspended in 40 mM Tris-HCl pH 7.4 buffer at a concentration corresponding to 50 mg original tissue/ml.

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-^{3}H]spiroperidol (23 Ci/mmol). The amount of tissue used per tube corresponded to 5–10 mg original wet weight and contained 300–400 μg protein as determined by the method of Lowry et al.[18]. At the end of 15 min incubation at 37 °C samples were filtered on glass fiber discs (25 mm diameter, 0.3 μm 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-DL-[benzilic 4,4-^{3}H]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-^{3}H]naloxone (50 Ci/mmol) with 10^{-6} M levorphanol as a competitor. In the case of this latter assay, the incubation temperature was 25 °C and this was preceded by a preincubation (30 min, 37 °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 I

Effect of kainate or acrylamide on retinal peptides of the chick

Each bird was monocularly injected with either 27 μg kainate or 50 μg acrylamide. Values represent a mean derived from 6–9 birds, together with the standard error. See text for further details.

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

* $P < 0.05$ 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[7–9,16,32,34] it is surprising that neurotensin levels remain unchanged after kainate treatment. This may reflect a differential sensitivity to kainate of the various amacrine populations involved. Such varying sensitivity has been suggested for CNS neurons[23]. 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[27] an effect perhaps mediated by the impairment of axoplasmic transport, reported in the chicken by Souyri et al.[31]. Such distal effects may be direct or consequent to abnormal protein synthesis in the nerve cell body[28]. 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[2].

There has been discussion as to whether the neurotoxic effects of acrylamide are mediated by the formation of acrylamide metabolites by the liver[1]. 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[13]. 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 [^{3}H]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[1], and suggests a lack of interdependence of dopaminergic and enkephalin-related circuitry. The retinal dopamine receptor closely resembles its counterpart in the brain[19], and dopamine is the principal catecholamine of retina[20]. However, spiroperidol is also known to bind to serotonergic receptors[24]. 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[14].

TABLE II

Effect of kainate or acrylamide on retinal receptor sites

Data are expressed as pmol bound/g membrane protein ± S.E. Each data point was derived from 6–14 birds. Incubation conditions are described in the text.

Labeled ligand	*Control*	*Acrylamide*	*Kainate*
QNB	59 ± 2	61 ± 11	37 ± 7*
Naloxone	7.7 ± 0.6	19.5 ± 1.2*	8.6 ± 1.3
Spiroperidol	1085 ± 143	1113 ± 75	1199 ± 69

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

The increased retinal Met-enkephalin found after acrylamide treatment was concomitant with a parallel increase in the binding of [^{3}H]naloxone (Table II). However, the decreased level of this peptide seen in kainate-treated retina did not coincide with a changed level of [^{3}H]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, compatable 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 [^{3}H]QNB binding (Table II). A preliminary report of a similar change in kainate-treated rat retina has appeared[26]. 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[4].

REFERENCES

1 Agrawal, A. K., Seth, P. K., Squibb, R. E., Tilson, H. A., Uphouse, L. L. and Bondy, S. C., Neurotransmitter receptors in brain regions of acrylamide treated rats. I. Effects of a single exposure to acrylamide, *Pharmacol. Biochem. Behav.*, 14 (1981) 527–531.

2 Auld, R. B. and Bedwell, S. F., Peripheral neuropathy with sympathetic overactivity from industrial contact with acrylamide, *Canad. Med. Ass. J.*, 96 (1967) 652.

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.

4 Bjorklund, H., Hoffer, B., Olson, L. and Seiger, A., Differential morphological changes in sympathetic nerve fibers elicited by lead, cadmium, and mercury, *Environ. Res.*, 26 (1981) 69–80.

5 Bondy, S. C., Neurotransmitter binding interactions as a screen for neurotoxicity. In A. Vernadakis and K. N. Prasad (Eds.), *Mechanisms of Neurotoxic Substances*, Raven Press (1982) pp. 25–50.

6 Bondy, S. C., Rapid screening of neurotoxic agents by in vivo and in vitro means. In R. M. Gryder and V. H. Frankos (Eds.), *Effects of Food and Drugs on the Development and Function of the Nervous System*, U.S. Government Printing Office, 1980, pp. 133–143.

7 Brecha, N., Karten, H. J. and Laverack, C., Enkephalin-containing amacrine cells in the avian retina: immunohistochemical localisation, *Proc. nat. Acad. Sci. (U.S.A.)*, 76 (1979) 3010–3014.

8 Brecha, N., Karten, H. J. and Schenker, C., Neurotensin-like and somatostatin-like immunoreactivity within amacrine cells of the retina, *Neuroscience*, 6 (1981) 1329–1340.

9 Buckerfield, M., Oliver, J., Chubb, I. W. and Morgan, I. G., Somatostatin-like immunoreactivity in amacrine cells of the chicken retina, *Neuroscience*, 6 (1981) 689–695.

10 Fratta, W., Yang, H. Y. T., Majane, B. and Costa, E., Distribution of β-endorphin and related peptides in the hypothalamus and pituitary, *Neuroscience*, 4 (1979) 1903–1908.

11 Govoni, S., Hong, J. S., Yang, H. Y. T. and Costa, E., Increase of neurotensin content in nucleus accumbens by haloperidol treatment, *J. Pharmacol. exp. Therap.*, 215 (1980) 413–417.

12 Hong, J. S., Yang, H. Y. T. and Costa, E., Substance P content of substantia nigra after chronic treatment with antischizophrenic drugs, *Neuropharmacology*, 17 (1978) 83–86.

13 Hong, J. S., Yang, H. Y. T., Fratta, W. and Costa, E., Rat striatal Met-enkephalin content after chronic treatment with cataleptogenic and non-cataleptogenic antischizophrenic drugs, *J. Pharmacol. exp. Therap.*, 205 (1978) 141–147.

14 Howells, R. D., Groth, J., Hiller, J. M. and Simon, E. J., Opiate binding site in the retina: properties and distribution, *J. Pharmacol. exp. Therap.*, 215 (1980) 60–64.

15 Imperato, A., Porceddu, M. L., Morelli, M., Fossarello, M. and Di Chiara, G., Benzodiazepines present kainate-induced loss of GABA-ergic and cholinergic neurons in chick retina, *Brain Research*, 213 (1981) 205–210.

16 Karten, H. J. and Brecha, N., Localisation of substance P immunoreactivity in amacrine cells of the retina, *Nature (Lond.)*, 283 (1980) 87–88.

17 Laneralle, N. C., Elde, R. P., Sparber, S. C. and Frick, M., Distribution of methionine-enkephalin immunoreactivity in the chick brain: an immunohistochemical study, *J. comp. Neurol.*, 199 (1981) 513–533.

18 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with the Folin phenol reagent, *J. biol. Chem.*, 193 (1951) 265–275.

19 Magistretti, P. J. and Schorderet, M., Dopamine receptors in bovine retina: characterization of the ^{3}H-spiroperidol binding and its use for screening dopamine receptor affinity of drugs, *Life Sci.*, 25 (1979) 1675–1686.

20 Makman, M. H., Dvorkin, B., Horowitz, S. G. and Thal, L. J., Properties of dopamine agonist and antagonist binding to sites in mammalian retina, *Brain Research*, 194 (1980) 403–418.

21 McGeer, E. F., Olney, J. W. and McGeer, P. L. (Eds.), *Kainic Acid as a Tool in Neurobiology*, Raven Press, New York, 1978.

22 Morgan, I. G. and Ingham, C. A., Kainic acid affects both plexiform layers of chicken retina, *Neurosci. Lett.*, 21 (1981) 275–280.

23 Nadler, J. V., Kainic acid: neurophysiological and neurotoxic actions, *Life Sci.*, 24 (1979) 289–300.

24 Peroutka, S. J. and Snyder, S. H., Multiple serotonin receptors: differential binding of ^{3}H-5 hydroxytryptamine, ^{3}H-lysergic acid diethylamide and ^{3}H-spiroperidol, *Mol. Pharmacol.*, 16 (1979) 687–699.

25 Pert, C. B. and Snyder, S. H., Opiate receptor: demonstration in nervous tissue, *Science*, 179 (1973) 1011–1014.

26 Redburn, D. A., Mitchell, C. K., Samson, F. E. and Gomez-Ramos, P., Differential effects of kainic acid on muscarinic and nicotine receptors, *Trans Amer. Soc. Neurochem.*, 13 (1982) 103.

27 Schaumberg, H. H. and Spencer, P. S., Environmental hydrocarbons produce degeneration in cat hypothalamus and optic tract, *Science*, 199 (1978) 199–200.

28 Schotman, P., Gipon, L., Jennekens, F. G. I. and Gispen, W. H., Polyneuropathies and CNS protein metabolism. III. Changes in protein synthesis induced by acrylamide intoxication, *J. Neuropathol. exp. Neurol.*, 37 (1978) 820–837.

29 Schwarcz, R. and Coyle, J. T., Kainic acid: neurotoxic effects after intraocular injection, *Invest. Ophthalmol.*, 16 (1977) 141–148.

30 Schwarcz, R., Fuxe, K., Agnati, L. F., Hökfelt, T. and Coyle, J. T., Rotational behaviour in rats with unilateral striatal kainic acid lesions: a behavioral model for studies on intact dopamine receptors, *Brain Research*, 170 (1979) 483–495.

31 Souyri, F., Chretien, M. and Droz, B., 'Acrylamide-induced' neuropathy and impairment of axonal transport of proteins. I. Multifocal retention of fast transported proteins at the periphery of axons as revealed by light microscopic radioautography, *Brain Research*, 205 (1981) 1–13.

32 Stell, W., Marshak, D., Yamada, T., Brecha, N. and Karten, H., Peptides are in the eye of the beholder, *Trends Neurosci.*, 3 (1980) 292–295.

33 Willow, M. and Morgan, I. G., Retinal benzodiazepine receptors are destroyed by kainic acid lesions, *Neurosci. Lett.*, 20 (1980) 147–152.

34 Yamada, T., Marshak, D., Basinger, S., Walsh, J., Moreley, J. and Stell, W., Somatostatin-like immunoreactivity in the retina, *Proc. nat. Acad. Sci. U.S.A.*, 77 (1980) 1691–1695.