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Axonal Transport of Macromolecules
II. Nucleic Acid Migration in the Central Nervous System

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Summary. The axonal migration of nucleic acids and their precursors has been studied following injection of radioactive uridine or thymidine into a single eye of newhatched chicks. After monocular injection of tritiated uridine, a progressive increase was observed in the specific activity of RNA of the optic lobe contralateral to the injected eye. This accumulation was first apparent 18 hours after injection and increased for at least 6 days. There was also a lesser accumulation of trichloracetic acid soluble radioactivity in this contralateral lobe relative to the ipsilateral lobe. RNA was prepared from morphological fractions of optic lobes after differential centrifugation. Radioactive analysis of these RNA fractions suggested that mitochondrial, ribosomal and transfer RNA all migrated axonally to a considerable degree. At neither 4 hours or 4 days after monocular injection of tritiated thymidine, was there any excess of radioactivity in DNA of lobes contralateral to the injected eye relative to DNA of ipsilateral lobes. Thus there was no evidence for the flow of DNA along the axon. The data suggest that a wide variety of RNA species synthesised in the nerve cell body migrate distally and appear at nerve endings.

Key Words: Axon flow — Nucleic acids — Transport — Avian visual system — Optic nerve

Introduction

While the axoplasmic transport of protein has been well established, it is only recently that the axonal flow of ribonucleic acid has been investigated (Bray and Austin, 1968; Casola, Davis and Davis, 1969). Ribosomal RNA may be within the axon (Edstrom *et al.*, 1962; Koenig, 1965; Edstrom *et al.*, 1969), and the presence of RNA within synaptosomes has been reported (Austin and Morgan, 1967). What proportion of this RNA is due to axon flow from the perikaryon rather than localized mitochondrial RNA synthesis is not known.

This report is a study of the velocity of the axonal transport of nucleic acids and of the types of molecule transported. The avian visual system has been used as this is a useful model for such studies, as has been described in the previous paper (Bondy, 1971).

Experimental

Day old chicks of white Leghorn strain Kl 37 were intraocularly injected with 10 μl of an aqueous solution of either (5-^{3}H)-uridine (27.1 C/μ-mole) or (methyl-3-^{3}H)-thymidine (0.36 C/m-mole) containing 10 μC or 7.5 μC respectively. Chicks were lightly anesthetized with fluothane and either the left or the right eye was

injected using a 1/2'' No. 30 gauge hypodermic needle. After various intervals chicks were killed by decapitation and optic lobes and cerebral hemispheres dissected out on ice. The specific activity of RNA or DNA and the level of unincorporated radioactivity was determined in these brain regions.

Ribonucleic Acid Assay

Brain regions or fractions of regions, from chicks injected with ^{3}H-uridine were homogenized in 5 ml cold 5% trichloracetic acid and centrifuged at 0°C at 3000 g for 10 min. The supernatant constituted the acid soluble nucleotide precursor pool and 1-ml portions were counted in 10 ml standard scintillation solution. The precipitates were washed at 0°C successively in 5% trichloracetic acid (twice), ethanol (twice), and once with ether. The final precipitates were suspended in 1.25 ml 0.3 N KOH for 2 hours at 37°C. This solution was then neutralized with 70% perchloric acid and the resulting protein, DNA and $KClO_4$ centrifuged down at 0°C. Aliquots of the supernatants were taken for colorimetric assay of RNA (Ceriotti, 1955) and 0.5 ml portions were added to 10 ml of standard scintillation solution. Standard scintillation solution consisted of a 2:10 (v/v) solution of Beckman Bio-Solv solubilizer No. 3 and toluene, containing 0.4% 2-phenyl-5-(biphenyl-2-yl)-1, 3, 4 oxidiazole and 0.008% 1—4 bis (5-phenyloxazole-2-yl) benzene. Radioactivity was measured in a Packard Tri-Carb scintillation spectrometer at an efficiency of 28%. Instrument background was 15 counts/min.

Deoxyribonucleic Acid Assay

Optic lobes from chicks injected with ^{3}H-thymidine were homogenized in cold 5% trichloracetic acid and the acid soluble supernatant recovered and counted as for the RNA assay. The precipitate was washed, incubated with 0.3 N KOH and neutralized with perchloric acid as described in the RNA assay. The precipitate of protein, DNA and $KClO_4$ was then heated with 2.5 ml 0.5 N $HClO_4$ for 20 min at 90°C in order to hydrolyse the DNA. After cooling, the mixture was centrifuged and aliquots of the supernatant were taken for the colorimetric determination of DNA by a diphenylamine-based method (Burton, 1956). One ml fractions of the supernatant were mixed with 15 ml standard scintillation solution and counted (described above).

Morphological Fractionation

Each chick optic lobe was homogenized in 3.5 ml 0.32 M sucrose and the homogenate centrifuged at 1000 g for 10 min. The resulting pellet was rejected. The supernatant was centrifuged at 25000 g for 15 min, the resulting precipitate constituting the mitochondrial fraction. When examined under a phase contrast microscope, this fraction was shown devoid of intact cells, nuclei or erythrocytes. The supernatant from this was centrifuged at 100,000 g for 60 min. This yielded a ribosomal pellet which contained no microscopically apparent mitochondria. The final supernatant constituted the soluble fraction.

Each data point presented is the mean of at least four experimental birds, separately analysed. Regions contralateral to and directly innervated by the injected eye are referred to as C, while ipsilateral regions are referred to as I. Probability of the ratio of paired sets of data being greater than unity was calculated by Students' one-tailed T-test.

Results

At one and at 6 hours after monocular injection of 10 μC ^{3}H-uridine, there was no significant difference in the specific activity of RNA within paired optic lobes (Table 1). At later times, a progressive asymmetry became apparent, increasing for at least 6 days. This implied a gradual movement of RNA distally along the axon. The rate of migration of RNA was slower than that of protein in a precisely parallel system (Bondy, 1971). Casola *et al.* (1969) reported axonal RNA flow to be relatively slow although they reported a more rapid flow (or directional diffusion) or RNA precursors along the axon (see also Austin *et al.*, 1966). In our system,

Table 1. *Incorporation of radioactivity into RNA of paired optic lobes after monocular injection of 10 μC(5-^{3}H)-uridine (21.7 C/m-mole)*

C = lobe contralateral to injected eye
I = lobe ipsilateral to injected eye

Time after injection	Acid soluble			RNA bound		
	Counts/min/10 mg tissue			Counts/min/mg RNA		
	C	I	C/I	C	I	C/I
1h	370	381	0.97 ± 0.03	510	520	0.98 ± 0.04
6h	380	359	1.05 ± 0.04	1,370	1360	1.01 ± 0.03
18h	1490	980	1.52 ± 0.18*	3,720	1840	2.02 ± 0.31*
3d	560	455	1.23 ± 0.15	12,550	1880	6.68 ± 1.2*
6d	—	—	—	20,300	2020	10.05 ± 1.7*

* $P < 0.05$

Table 2. *Incorporation of radioactivity into RNA in fractions of optic lobes, 2 days after monocular injection of 10 μC(5-^{3}H)-uridine (21.7 C/m-mole)*

C and I = as in Table 1

Fraction	Counts/min/mg RNA		
	C	I	C/I
Mitochondrial	6620	2470	2.67 ± 0.54*
Ribosomal	7280	2320	3.14 ± 0.51*
Soluble	7390	2180	3.34 ± 0.40*

* $P < 0.05$

no early asymmetry within the acid soluble pool of paired optic lobes was observed, but a difference of acid soluble pool radioactivity within optic lobes appeared at 18 hours after injection. This was probably no longer due to direct RNA precursors. This soluble radioactivity was not sufficient to account for the major asymmetry subsequently observed in the RNA compartment.

At no time was a significant differential observed between paired cerebral hemispheres. As avian cerebral hemispheres receive direct innervation from the ipsilateral optic lobes and not from the optic nerves, this suggests that major transneuronal migration of RNA species or oligonucleotides does not occur.

Two days after monocular injection of ^{3}H-uridine, a morphological fractionation of optic lobes was carried out and the specific activity of RNA within these fractions determined. RNA within the soluble, mitochondrial and ribosomal fractions, all appeared to migrate along the axon (Table 2). As the asymmetry between the contralateral and ipsilateral lobes was similar in all cases, the rate of RNA transport in the 3 fractions appeared to be similar.

Four hours and three days after injection of 7.5 μC ^{3}H-thymidine into a single eye, chick optic lobes were dissected out and the specific activity of their constituent DNA determined. At neither time was a significant difference observed between paired optic lobes (Table 3). Also, no evidence was found for the axonal migration of soluble DNA precursors.

Table 3. *Incorporation of radioactivity into DNA of paired optic lobes after monocular injection of 7.5 μC(methyl-3-^{3}H)-thymidine (0.36 C/m-mole)*

C and I = as in Table 1

Time after injection	Acid soluble			DNA bound		
	Counts/min/10 mg tissue			Counts/min/mg DNA		
	C	I	C/I	C	I	C/I
4h	925	919	1.01 ± 0.03	3780	3870	0.98 ± 0.02
3d	—	—	—	11,250	11,840	0.95 ± 0.03

Discussion

A large part of the RNA migrating along the axon has been reported as being within the mitochondrion (Edstrom *et al.*, 1969) which may have its origin in the nerve cell body (Barondes, 1966). Although we have evidence for mitochondrial RNA transport distally along the axon, we have not detected any such movement of DNA. This may be due to the relatively higher rate of mitochondrial RNA synthesis (Kalf and Ch'ih, 1968; Suyama and Eyer, 1968).

A rapid flow of microsomes along the axon has been reported (Sjostrand, 1970). A problem is that free ribosomes have not been found at the presynaptic terminal or within the axon (Palay and Palade, 1955). However the presence of ribosomal RNA in axons has been described (Casola *et al.*, 1969; Edstrom and Sjostrand, 1969). The precise location of this RNA is uncertain. Peterson *et al.* (1968) suggest that this RNA is largely in Schwann cells surrounding axons. Amaldi and Rusca (1970) using cultured sympathetic ganglia, reported RNA to be within axons that were free of surrounding glial cells.

Our data suggest that a considerable axonal migration of RNA takes place distally from the nerve cell body. This flow may involve the transport of soluble and particulate RNA. Ribosomal RNA appears to be transported, although its destination and precise localization are unknown.

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