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Authors

Margolis, TP
Marchand, CM
Kistler, HB
et al.

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Uptake and Anterograde Axonal Transport of Wheat Germ Agglutinin from Retina to Optic Tectum in the Chick

TODD P. MARGOLIS, CLAUDINE M.-F. MARCHAND, HENRY B. KISTLER, JR., and JENNIFER H. LAVAIL
Department of Anatomy, University of California, San Francisco, California 94143

ABSTRACT The uptake and anterograde axonal transport of ^{125}I-wheat germ agglutinin (WGA) has been investigated in the visual system of the chick. In order to obtain a marker with specific and homogeneous binding properties, the iodinated lectin was affinity purified by passage over an *N*-acetylglucosamine (NAcGlu)-Sepharose column after iodination. 22 h after vitreal injection of the purified ^{125}I-WGA, radioactive label was found accumulated in the retinoreceptive layers of the contralateral optic tectum. Gel electrophoresis of tectal homogenates revealed that > 80% of the retrieved label ran in a band which comigrated with native WGA. In chicks injected with the fraction of the iodinated preparation that failed to bind to the affinity column, there was no evidence of tectal labeling. These findings support the hypothesis that WGA is selectively taken up by chick retinal ganglion cells and transported intact in an anterograde direction to their axon terminals in the contralateral optic tectum. This raises the possibility that constituents of perikaryal membrane, i.e., lectin receptors, are transported in an anterograde direction by chick retinal ganglion cells.

Wheat germ agglutinin (WGA) belongs to a class of proteins known as lectins, that bind specific carbohydrate moieties in much the same way that antibodies bind antigens. Due to its particularly high affinity for *N*-acetylglucosamine (NAcGlu) and sialic acid, WGA has been widely used as a probe for the recognition of membrane glycoproteins and glycolipids containing these sugar groups (1). More recently, it has been useful as a marker for axonal transport. Thoenen and his colleagues (2) originally opened this avenue by their important observations that the lectin was transported in a retrograde direction, i.e., from axon endings back to neuronal cell bodies. It accumulated in rat superior cervical ganglion cells after its introduction into the anterior eye chamber (2). Since then, they and others have extended the finding to descriptions of lectin-membrane binding characteristics (3) and ultrastructural localization of the probe in cell bodies after retrograde axonal transport (4, 5).

In addition, other workers have recently found that the lectin may be transported in a retrograde direction by some neurons and, coincidentally but less reliably, in an anterograde direction by other neurons (6–8). In these cases of anterograde transport, relatively high concentrations of WGA have been injected, and no effort has been made to determine either the cellular mechanism of uptake or whether the lectin or breakdown products were transported.

In light of the specific binding properties of WGA, and the insight this might give us into the cellular processes of axonal transport and membrane reutilization, we have begun a systematic study of the uptake and anterograde transport of WGA. We present here evidence that, after vitreal injection, affinity-purified, iodinated WGA is selectively taken up by chick retinal ganglion cells and transported intact in the anterograde direction to axon terminals in the contralateral optic tectum. If the lectin remains associated with components of the perikaryal membrane during transport, this raises the possibility that portions of this membrane, i.e., lectin "receptors", may be circulated within the neuron from cell body to axon terminal.

MATERIALS AND METHODS

Animals

We used 1- to 5-d-old White Leghorn chicks in all of our studies. The advantages of studying transport in the chick visual system are three-fold. First, the completely crossed projection of retinal ganglion cell axons to the opposite optic tectum enables us to study anterograde transport of radioactively labeled WGA by use of the ipsilateral optic tectum as an internal control. Second, the completely uncrossed projection of the superior cervical ganglion cell to the iris of the eye allows us to study the retrograde transport of the radioactive label in the same animal while we are studying anterograde transport to the tectum. Lastly, by vitreal injection, the retinal ganglion cell bodies can be exposed to the lectin with a minimum of pathological insult. Thus, we are able to study the uptake and transport of ^{125}I-WGA in a relatively undisturbed system.

Preparation of the Iodinated Proteins

WGA was iodinated using a modification of the chloramine-T method of Hunter and Greenwood, as described by Burridge (9). WGA (2.5 mg/ml, Sigma Chemical Co., St. Louis, Mo.) and NAcGlu (5 mg/ml, Sigma Chemical Co.) were combined in 200 μl of 0.2 M phosphate buffer (pH 7.4). 2 mCi of carrier-

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free ^{125}I-Na in 0.1 N NaOH (New England Nuclear, Boston, Mass.) and 10 μl of chloramine-T (2.5 mg/ml, Sigma Chemical Co.) were then added. After 2–10 min, the reaction was stopped by the addition of 20 μl of Na metabisulfite (12.5 mg/ml, Sigma Chemical Co.), and iodinated lectin was separated from unreacted iodine and NAcGlu by exclusion chromatography on a Bio-Gel P-2 column (Bio-Rad Laboratories, Richmond, Calif.). An aliquot of this preparation was reserved (fraction A). The remainder of the preparation was adjusted to pH 4.4 with 1.0 M Na acetate buffer and purified by affinity chromatography on a NAcGlu-Sepharose column (10, 11). The affinity purification yielded two fractions of iodinated protein: fraction B (nonspecific) that did not bind to the column and was eluted with 50 mM Na acetate buffer (pH 4.4), and fraction C (specific) that bound to the column. This fraction C was subsequently eluted from the affinity column with 1 M NAcGlu and dialyzed extensively against 0.1 M phosphate buffer (pH 7.4) at 4°C. SDS gel electrophoresis and subsequent autoradiography of the gel served to characterize the specific and nonspecific fractions. The autoradiograms were analyzed with an E. C. Apparatus Corp. densitometer (St. Petersburg, Fla.) To determine the specific activities of the iodinated lectin, we counted TCA precipitates of aliquots in a Beckman Biogamma counter, 70% efficiency (Beckman Instruments, Inc., Electronic Instruments Div., Schiller Park, Ill.), and analyzed protein concentration, according to the method of Hartree (12). Bovine serum albumin (BSA; Sigma Chemical Co.) was iodinated in a similar manner, but without NAcGlu in the reaction mixture. As with WGA, the reaction product was purified on a Bio-Gel P-2 column, but it was not subjected to affinity chromatography.

Axonal Transport Studies

Within 5 d of its preparation, a sample of 0.5–1.25 μCi of fraction C, the affinity-purified ^{125}I-WGA (0.25 μCi/μg sp act), in 25 μl of 0.1 M phosphate buffer (pH 7.4), was injected into the vitreal chamber through a hole made a few seconds earlier in the scleral margin of one eye of each of nine chicks. Assuming a vitreal volume of ~140 μl (13), this supplied about a 5×10^{-7} M concentration of iodinated WGA to the retina. After 21–23 h, the animals were reanesthetized and perfused intracardially with buffer and fixative as described previously (14). The dissected eyes, brains, and superior cervical ganglia from these animals were embedded in paraffin, and 8–10-μm thick sections of these tissues were prepared for autoradiography. 6 wk later the emulsion-coated slides were developed and analyzed by light microscopy. As controls, 2 μCi of fraction A (0.2 μCi/μg sp act) was injected into the vitreal chamber of one eye of five chicks, and 1.0–1.8 μCi of fraction B (0.6 μCi/μg sp act) was similarly injected into one eye of five other chicks. Additionally, 9.0–19.0 μCi of ^{125}I-BSA was injected into four chicks. Tissues from these animals were processed and analyzed in the same manner as those injected with the affinity-purified ^{125}I-WGA.

In additional studies, an aliquot of 8.0–10.0 μCi of affinity-purified ^{125}I-WGA (fraction C; 0.75 μCi/μg sp act) in 20 μl of 0.1 M phosphate buffer (pH 7.4), was injected into the vitreal chamber of one eye of all six chicks. After 22 h, the animals were perfused with phosphate-buffered saline for 3–5 min, and the tecta from four of the animals were quickly dissected, counted in a gamma counter, and individually homogenized in 200 μl of 5 mM Tris-HCl (pH 7.4) with 0.1% SDS. The homogenates were freeze-thawed repeatedly, heated to 100°C for 2 min, and centrifuged at 20,000 *g* for 30 min. The resulting supernates were subjected to SDS discontinuous gel electrophoresis (0.1% SDS, 15% polyacrylamide), as described by Laemmli (15). Gels were stained with 0.1% Coomassie Blue, dried onto filter paper, and exposed to Kodak X-Omat film for 10 d at −70°C, using a Dupont Image Intensification Screen (E. I. Dupont, DeNours and Co., Wilmington, Del.). The resulting autoradiograms were analyzed with a densitometer. Cytochrome *C*, carbonic anhydrase, phosphorylase *a*, BSA, IgG heavy chain, native WGA, and ^{125}I-WGA were all used as molecular weight standards. The tecta of the other two animals were counted only in a gamma counter.

RESULTS

Anterograde Transport

The ganglion cell, outer plexiform, and optic fiber layers of the retinas of all nine chicks injected with the affinity-purified ^{125}I-WGA (fraction C) were heavily labeled with silver grains. In all nine animals, heavy labeling was found in the contralateral optic tectum (Fig. 1 *a*) over the axons of the *Stratum opticum* (SO), and extending from layer *a* through layer *f* of the *Stratum griseum et fibrosum superficiale* (SGFS), the retinoreceptive layers of the optic tectum (16) (Fig. 2). Layer *d* appeared more heavily labeled, and layers *c* and *f* were less heavily labeled than other layers. The particularly heavy labeling of layer *d* was consistent with the finding of heavy labeling of this layer after vitreal injections of radioactive leucine or fucose (17, 18). There was no label above background over the ipsilateral optic tecta of these animals (Fig. 1 *b*).

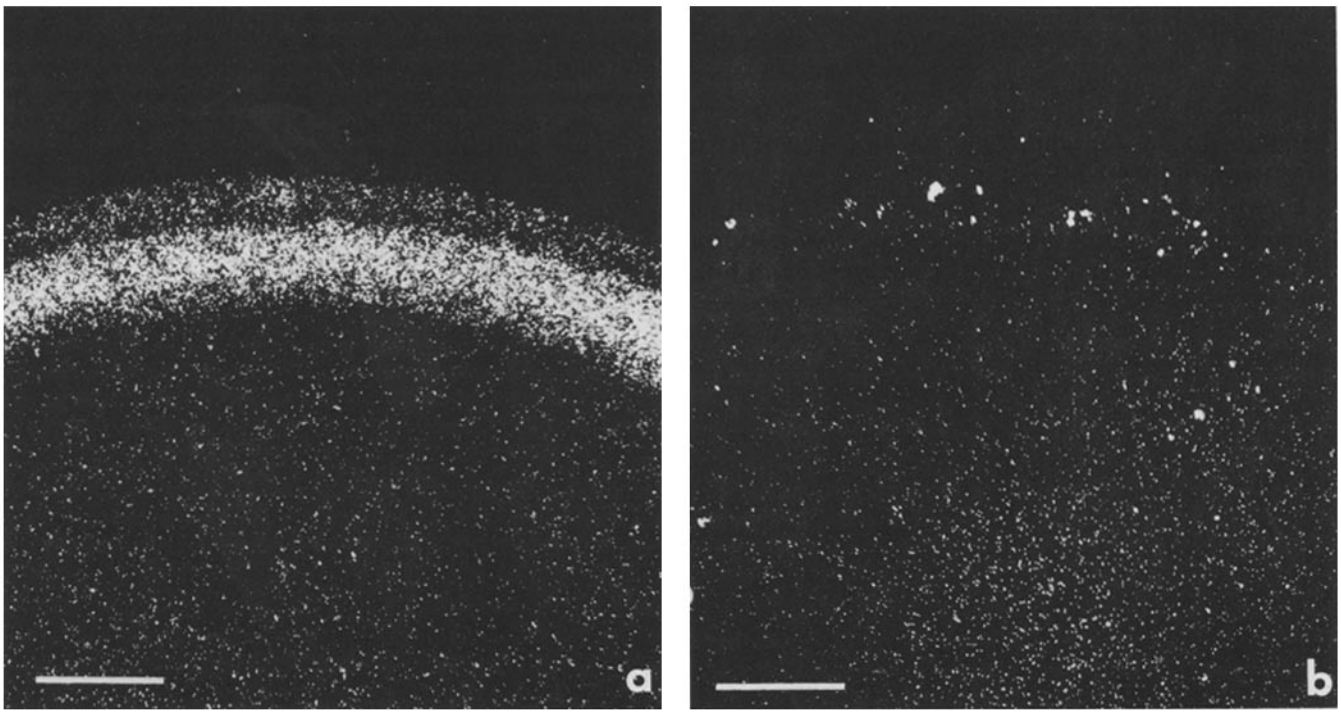

FIGURE 1 Dark-field micrographs of autoradiograms of the optic tecta of a chick injected vitreally with affinity-purified ^{125}I-WGA. The optic tectum contralateral to the injected eye (*a*) contains radioactive label over the SO and layers *a* through *f* of the SGFS. The ipsilateral optic tectum (*b*) was unlabeled. Bars, 100 μm. × 170.

Following intravitreal injections of the unpurified preparation of iodinated lectin (fraction A), we found results similar to those obtained with the affinity-purified lectin, i.e., the contralateral optic tectum was labeled, but the ipsilateral optic tectum contained no label above background. By contrast, in five chicks that received vitreal injections of the nonspecific fraction B, neither optic tectum was labeled despite homogeneous labeling throughout the retina of the injected eye. Furthermore, the ^{125}I-BSA injected vitreally in four chicks also failed to label either optic tecta.

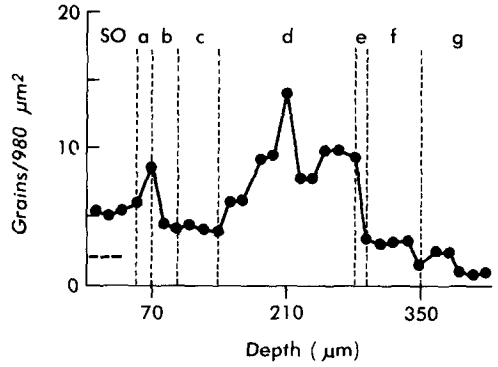

FIGURE 2 The density of labeling as a function of depth in the labeled optic tectum. The average number of grains is presented for six traverses across the autoradiogram. Layers *d* and *a-b* are more heavily labeled; layers *c* and *f* are less heavily labeled. The background level of radioactivity measured in adjoining midbrain tissue is indicated by the horizontal dashed line. The boundaries of the layers *a–f* are indicated by vertical dashed lines.

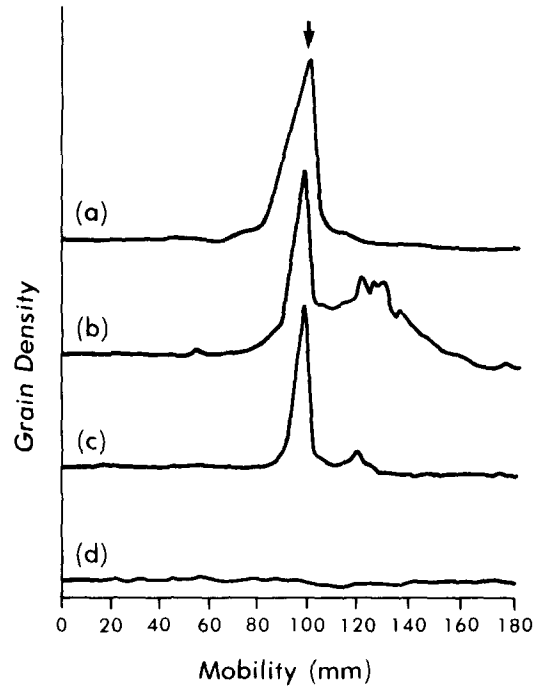

FIGURE 3 Densitometric tracings of autoradiograms of SDS gels. (*a*) Grain density of autoradiogram of affinity-purified fraction of ^{125}I-WGA. (*b*) Density of grains found over autoradiograms of the nonspecific fraction, B. (c) Density of grains found over autoradiogram of SDS gel of homogenates of the experimental optic tectum. (*d*) Density of grains found over autoradiogram of the gel of the control optic tectum. The arrow indicates the 18,000 dalton monomer of ^{125}I-WGA.

To determine whether the radioactive labeling of the tecta seen after injections of the affinity-purified lectin was due to intact ^{125}I-WGA rather than radioactive breakdown products, we recovered the radioactive label from the optic tecta of four chicks and characterized it by SDS polyacrylamide gel electrophoresis. Although multiple protein bands were retrieved from the experimental tecta of these animals, as revealed by Coomassie Blue staining, densitometer measurements (Fig. 3 *c*) of the autoradiograms of these gels (Fig. 4, column *c*) revealed that >80% of the retrieved radioactive protein ran in a single band that comigrated with both native WGA and ^{125}I-WGA. In two animals, a second, fainter band at 12,000 daltons was also seen. This represented <15% of the recovered radioactivity and may represent a breakdown product of the iodinated lectin. Although Coomassie Blue staining of proteins from the control tecta was similar to that of the experimental tecta, no radioactive bands were seen in the autoradiograms of these gels (Fig. 3 *d*; Fig. 4, column *d*).

These autoradiographic and biochemical findings were further supported by assays of the total radioactivity present in the tecta of six animals vitreally injected with affinity-purified lectin. On the average, the experimental tecta contained about 10 times the radioactivity of the control tecta (7,747 ± 760 dpm vs. 761 ± 310 dpm, $n = 6$). Using these values, we estimate the amount of transported ^{125}I-WGA in the tectum 21–23 h after injection to be about 0.1 pM.

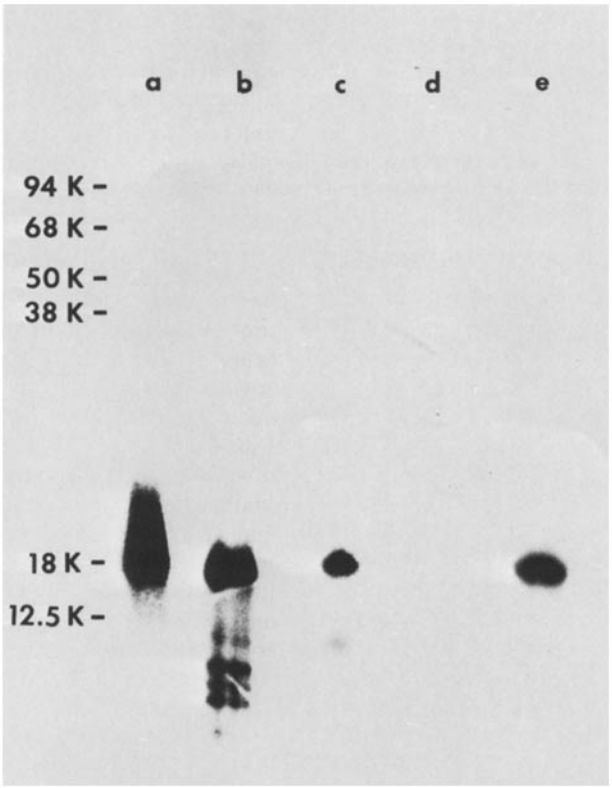

FIGURE 4 Composite SDS gel patterns: *a–d*, autoradiograms of the gels; *e*, Coomassie Blue staining pattern. The affinity-purifed fraction (*a*) contained a single radioactive band that comigrated with both the cold 18,000 dalton monomer of WGA (*e*) and the major radioactive band recovered after anterograde transport to the experimental tectum (*c*). No radioactive bands were observed in gels of the control tectum (*d*). The nonspecific fraction (*b*) consisted of multiple radioactive bands, the strongest of which comigrated with WGA. Molecular weight standards are indicated on the left.

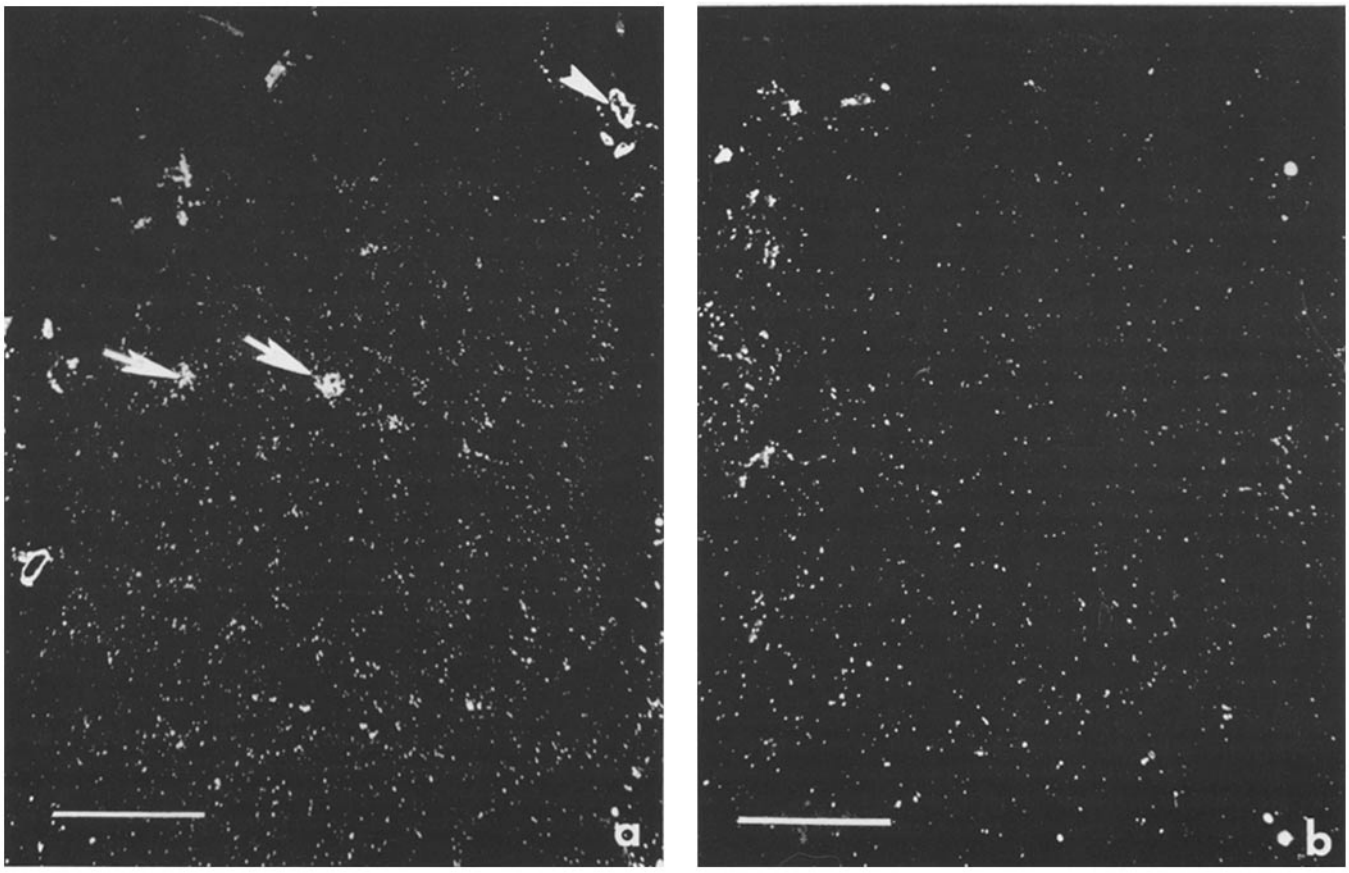

FIGURE 5 Dark-field micrographs of autoradiograms of the superior cervical ganglia after vitreal injection of the nonspecific fraction, B. Two labeled neuron cell bodies are indicated by arrows in the ipsilateral ganglion (*a*). Endothelial cells (arrowhead), glia, and pericytes were also found labeled in both the ipsilateral and contralateral ganglia × 133. No labeled neurons were found in the contralateral ganglia (*b*). Bars, 150 μm. × 100.

Retrograde Transport

In light of reports of the uptake and retrograde transport of labeled WGA from iris to the superior cervical ganglia in the rat, we examined the labeling of neuronal cell bodies in the chick superior cervical ganglion after vitreal injections. Despite heavy labeling of the irises of all animals, no labeled nerve cell bodies were found in the ipsilateral superior cervical ganglion of seven of the nine chicks injected with the affinity-purified ^{125}I-WGA. (In the remaining two animals, we failed to examine the ganglia.) We did, however, find labeled pericytes and endothelial cells in the ganglia of these animals. In contrast, in three out of five chicks injected with the nonspecific fraction (B), and in four out of five animals injected with the unpurified (fraction A) preparation, labeled nerve cell bodies were found in the ipsilateral (Fig. 5 *a*), but not the contralateral (Fig. 5 b) superior cervical ganglia.

DISCUSSION

Our observations support the hypothesis that ^{125}I-WGA is preferentially taken up and transported by retinal ganglion cell bodies to axon endings in the optic tectum. Although other investigators have reported a retrograde (4, 5) or bidirectional transport (6–8) of this lectin by neurons, ours is the first evidence that the selective affinity of WGA for specific carbohydrate moieties may play a role in its uptake and anterograde transport. Furthermore, ours is the first evidence that a fraction of the lectin avoids lysosomal degradation and is transported intact down the axon.

Despite equivalent or greater concentrations of the nonspecific fraction B (the one that failed to bind to the affinity column), of ^{125}I-BSA, or of ^{3}H-horseradish peroxidase,[1] only the fraction of WGA that retained its affinity for NAcGlu following iodination (fraction C) was taken up and transported in an anterograde direction by chick retinal ganglion cells. This observation supports our hypothesis of selective uptake and transport.

The affinity of ^{125}I-WGA for carbohydrate moieties of glycoproteins and glycolipids may thus play a role in the selective uptake of the lectin and its subsequent transport. In a fashion analogous to concanavalin A's induced patching and capping in lymphocytes (19), WGA may crosslink plasmalemmal glycoproteins or glycolipids and induce its endocytosis. Once inside the cell, a fraction of the ^{125}I-WGA is transported rapidly toward axon terminals. Although we have not systematically studied the rate of transport, assuming a minimum retinal ganglion cell axonal length of 15 mm (20) and a transit time of 22 h, it must be at least 16 mm/d. Given the rate of transport and the affinity of the lectin for glycoproteins, it is conceivable that the transport of the lectin is linked to the transport of endogenous glycoproteins. The anterograde transport of glycoproteins in retinal ganglion cells of developing chicks has been well documented (21).

[1] LaVail, J. H., and I. K. Sugino. Manuscript in preparation.

After injection of affinity-purified ^{125}I-WGA into four chicks, >80% of the radioactive protein we recovered from the experimental optic tecta appeared as a single radioactive band that comigrated with both native WGA and ^{125}I-WGA on SDS polyacrylamide gels. Because cells do not appear to reincorporate iodinated tyrosine into new protein (22), we considered this band to represent axonally transported ^{125}I-WGA. This observation extends the findings of Ruda and Coulter (6) concerning an immunologically identifiable fragment of the lectin in the optic pathway of rats following vitreal injections. If the WGA is not only intact, but remains associated with perikaryal membrane components after endocytosis and during transport, it raises the possibility that portions of this membrane may be circulated within the neuron, as has been recently proposed by Muller et al. (23) for plasma membranes of cultured macrophages.

Given earlier reports of the preferential uptake and retrograde axonal transport of WGA (4, 5), we also examined the labeling of neuronal cell bodies in the superior cervical ganglia after vitreal injections of the lectin. In contrast to these findings, we found no labeled neurons in the ipsilateral superior cervical ganglia in any of the animals injected with the affinity-purified ^{125}I-WGA. The differences between our results and those of others may be due to differences in species, methods of preparation, or concentrations of the WGA, survival times, or sites of injection (anterior chamber vs. vitreal chamber). However, it is relevant to note that we succeeded in labeling nerve cell bodies of these superior cervical ganglia in three out of five animals in which the nonspecific fraction (B) was injected. Thus, it seems likely that inactivated WGA, or some iodinated breakdown product, is responsible for the retrograde labeling reported here and may, at least in part, be responsible for the retrograde transport seen by others.

Not surprisingly, when we injected aliquots of the iodinated lectin that had not been passed over the affinity column (fraction A), we could identify both the anterograde transport of label to the contralateral optic tectum and the retrograde tranport of label to superior cervical ganglion cell bodies. Thus, our data suggest that the heterogeneity of tagged WGA molecues is responsible for the bidirectional transport observed by others (6–8).

This problem of heterogeneity was addressed by Dumas et al. (3), who depended on hemagglutination to assay for lectin binding activity after chloramine-T iodination. Such an assay, however, is relatively insensitive to slight changes in lectin binding activity. Because our nonspecific fraction (B) always represented <20% of the radioactive yield, the associated decrease in binding activity might easily have been undetected with a hemagglutination assay.

In summary, we have demonstrated the selective uptake and anterograde axonal transport of the membrane marker WGA. We believe that this extends the usefulness of WGA as a probe for the study of neuronal cell development, endocytosis, and membrane recycling. In addition, this study highlights the need for affinity purification of tagged lectins, as has already been emphasized in other systems by Sharon and Lis (1) and Maylié-Pfenninger and Jamieson (24).

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