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Fast axonal transport in auditory neurons of the guinea pig: A rapidly turned-over glycoprotein

(neuronal proteins/short axons/cochlea/spiral ganglion cells/protein transport)

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ABSTRACT **Proteins of the fast component of axonal transport were analyzed by one- and two-dimensional polyacrylamide gel electrophoresis in the guinea pig spiral ganglion, which has its cell bodies in the cochlea and its axons in the eighth cranial nerve projecting to the ipsilateral cochlear nucleus. We found that we could easily identify the proteins of the fast component even though these axons are only about 3 mm long because the cochlea minimized diffusion of labeled precursor into the cochlear nucleus. The composition of the fast component of the spiral ganglion cells was similar, but not identical, to the fast component of guinea pig retinal ganglion cells. One difference was the predominance in the spiral ganglion cell fast component of a rapidly turned-over glycoprotein (RTGP) with a molecular weight of 110,000–140,000 and an isoelectric point of 5.0. RTGP accumulated in the cochlear nucleus for just the first 3 hr after the application of the labeled precursor and then rapidly disappeared, whereas the other major fast component polypeptides continued to accumulate for 12–24 hr. RTGP was also tentatively identified in the fast component of retinal ganglion cells, but was not as prominently labeled relative to the other fast-component proteins in those cells. The rapid disappearance of RTGP from spiral ganglion cell terminals in the cochlear nucleus may be a result of secretion, perhaps as part of a synaptic vesicle, or retrograde transport as a feedback signal. The difference in the relative amounts of RTGP found in spiral ganglion and retinal ganglion cell terminals may reflect differences in the fundamental properties of the two groups of neurons.**

The characteristics of axonal transport have been derived almost exclusively from studies of relatively long axons, such as those in the sciatic and optic nerves (1). However, in the mammalian nervous system as a whole, most neurons have short axons. It is clearly important, then, to study neuronal systems that have neurons with short axons in order to determine how well the characteristics of axonal transport in long axons compare with those in short axons. The characteristics of transport that one might compare between long and short axons fall into two broad categories: (*i*) the rate of transport and (*ii*) the composition of each rate component. Although the rate of the fast and slow components can vary, as in central versus peripheral nervous system axons (2), the physiological significance of such differences is not clear. However, because different groups of neurons may secrete different substances, one is easily led to predict that these differences would be reflected by variations in some of the proteins composing the various rate components of axonal transport. The elucidation of such variations in axonally transported proteins will provide fundamental information about the molecular bases for specific structural and functional differences between groups of axons.

Axonal transport of proteins in short axons has not been extensively studied because of the problem caused by diffusion of the labeled precursor into the neuropil containing the axons and synaptic terminals of the target cell bodies. Large amounts of radioactive proteins may be locally synthesized in the neuropil a short distance from the site of application of the labeled precursor as the precursor diffuses. These locally synthesized proteins can interfere with the detection of axonally transported labeled proteins when the axons of the target cell bodies do not extend beyond the region of diffusion. Schubert and colleagues (3) have employed a method that circumvents this problem. They administer the precursor directly into the soma of a single neuron by iontophoresis. However, because only a small amount of precursor can be administered in this manner, their analyses have been limited to the autoradiographic localization of radioactive molecules; it has not been possible to analyze the individual molecules themselves. A short axon system in which the problem of precursor diffusion is considerably reduced, but that does permit analysis of individual proteins, is the spiral ganglion cell of the guinea pig cochlea. The cochlea serves as a container for the labeled precursor, thereby reducing diffusion considerably. Therefore, even though the cochlear nucleus containing the axon terminals of the spiral ganglion cells is only about 3 mm from the cochlea, labeling of locally synthesized proteins in the neuropil of the cochlear nucleus is negligible relative to the labeled axonally transported proteins. Our initial investigation of this system focused on the fast component proteins because they are involved in many of the dynamic processes taking place in the axon, such as transmitter storage and release and turnover of membrane (2). In the present study we analyze axonal transport in guinea pig spiral ganglion cells and describe a glycoprotein with a life span much shorter than any of the other major fast-component proteins.

METHODS

The exposed cochlea, auditory nerve, and cochlear nucleus are illustrated in Fig. 1. The cell bodies of the spiral ganglion cells are located in the bony core or modiolus of the cochlea. To expose those cell bodies to labeled precursor, the oval and round windows of the cochlea were visualized via a small hole cut through the temporal bone and membranes of the two windows removed. Small aliquots of the perilymph were then removed from the oval window and replaced via the round window with equal volumes of artificial perilymph (4) or phosphate-buffered saline containing about 100 or 125 μCi (1 Ci = 3.7×10^{10} becquerels) of labeled precursor in 15–20 μl until all the perilymph had been exchanged for the radioactive solution. After periods

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Abbreviations: BUST, 2% (vol/vol) β-mercaptoethanol/8 M urea/1% sodium dodecyl sulfate/0.1 M Tris·HCl, pH 7.3; RTGP, rapidly turned-over glycoprotein.

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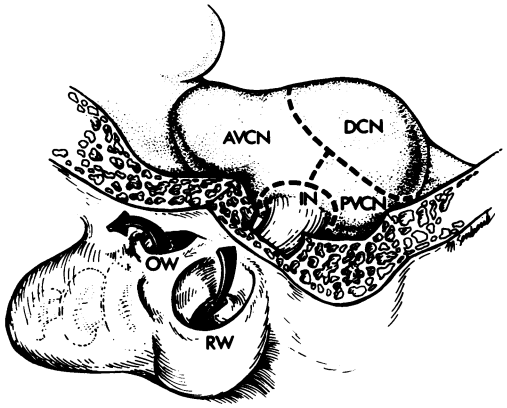

FIG. 1. Cochlea, auditory nerve, and the divisions of the cochlear nucleus (IN, interstitial nucleus; AVCN, anteroventral cochlear nucleus; DCN, dorsal cochlear nucleus; PVCN, posteroventral cochlear nucleus). The arrows indicate the removal of perilymph through the oval window (OW) and the infusion of labeled precursor through the round window (RW).

of 1–24 hr, the animals were sacrificed by decapitation and the auditory nerve and cochlear nucleus were removed. The tissue was prepared for analysis by homogenization in Laemmli solubilization buffer (5) or in a buffer consisting of 2% (vol/vol) β-mercaptoethanol, 8 M urea, 1% sodium dodecyl sulfate, and 0.1 M Tris·HCl, pH 7.3 (BUST). Samples of homogenate were subjected to sodium dodecyl sulfate/polyacrylamide gel electrophoresis on slab gels (5) and two-dimensional electrophoresis (6, 7). Labeled polypeptides in the gels were detected by fluorography (8, 9).

RESULTS

The labeled fast-component polypeptides were first examined in the axon terminals within the cochlear nucleus rather than in the nerve itself because they pass through the short nerve so rapidly. Fig. 2 illustrates the appearance of labeled polypeptides in the cochlear nucleus 1–24 hr after the administration of the labeled precursor. As early as 1 hr after injection, labeled polypeptides were detected in the cochlear nucleus, but most of the radioactivity was present in just two polypeptides with apparent molecular weights of 140,000 and 25,000. At 3–6 hr after injection, several more polypeptides of the fast component were observed, and by 24 hr after injection, the pattern became considerably more complex, reflecting the arrival of the more slowly moving polypeptides constituting the intermediate components [groups II and III of Willard *et al.* (10)] and slow component b (11). Of all the fast-component polypeptides, the 140,000-dalton one stood out because of its high specific activity and its kinetics. In Fig. 2, it is clear that the quantity of the intensely labeled 140,000-dalton polypeptide in the cochlear nucleus was maximal by 3 hr and that by 6 hr it had declined considerably. This kinetic pattern is quite different from that of the other polypeptides of the fast component, which continued to accumulate in the cochlear nucleus for 12 or more hr.

Because the 140,000-dalton polypeptide turned over so much more rapidly than the other fast-component polypeptides, it was studied in more detail. To determine if it was glycosylated, as many fast-component polypeptides are known to be (1), [^{3}H]fucose was used as the precursor in a kinetic analysis similar to that shown in Fig. 2. That analysis replicated what we had observed by using [^{35}S]methionine: the rapidly turned-over polypeptide was intensely labeled at early times after the administration of the [^{3}H]fucose and began to decline while the other glycoproteins of the fast component were still accumulating. An example of the pattern of labeled polypeptides at 2 hr after the [^{3}H]fucose was given is shown in Fig. 4*A*. It should be noted that in Fig. 4*A* the rapidly turned-over polypeptide has an apparent molecular weight of 110,000 rather than 140,000. This shift in apparent molecular weight seemed to be correlated with the addition of 8 M urea to the solubilization buffer used for this experiment. It is possible that the urea caused the polypeptide to become more denatured and bind more sodium dodecyl sulfate, resulting in the decline in apparent molecular weight (12, 13). To avoid confusion and emphasize those characteristics of the polypeptide that distinguish it from others in the fast component, we shall subsequently refer to it as the rapidly turned-over glycoprotein (RTGP) of the auditory system fast component.

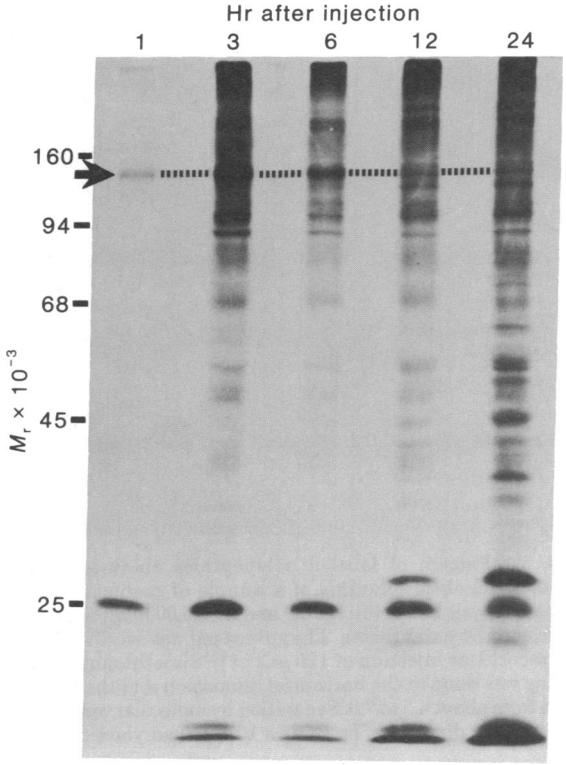

FIG. 2. The labeled polypeptides present in the cochlear nucleus at various times after administration of the labeled precursor. The arrow and broken line mark the position of the rapidly turned-over polypeptide. In each of five guinea pigs, one cochlea was perfused with 125 μCi of [^{35}S]methionine. The animals were sacrificed at the indicated times and the cochlear nucleus was removed from each and homogenized in Laemmli buffer (5). Samples of each homogenate were applied to adjacent wells of a 10% polyacrylamide gel and electrophoresed.

A few of the later-appearing fast-component polypeptides had apparent molecular weights that were similar to RTGP, so the high resolution of the two-dimensional gel electrophoresis technique was utilized to completely separate RTGP from those other polypeptides. The resulting fluorograph is illustrated in Fig. 3. The radioactive spot corresponding to RTGP was clearly resolved from several other labeled polypeptides with slightly lower apparent molecular weights. In addition, this analysis revealed that RTGP and the 25,000-dalton polypeptide each had an approximate pI of 5.0, making them two of the most acidic species that were detected in the fast component.

Because the auditory axons are short, a number of control experiments were performed to demonstrate that RTGP was axonally transported and was not a product of local reutilization of precursor in the cochlear nucleus. First, autoradiographs were made of the nucleus at several times after the intracochlear

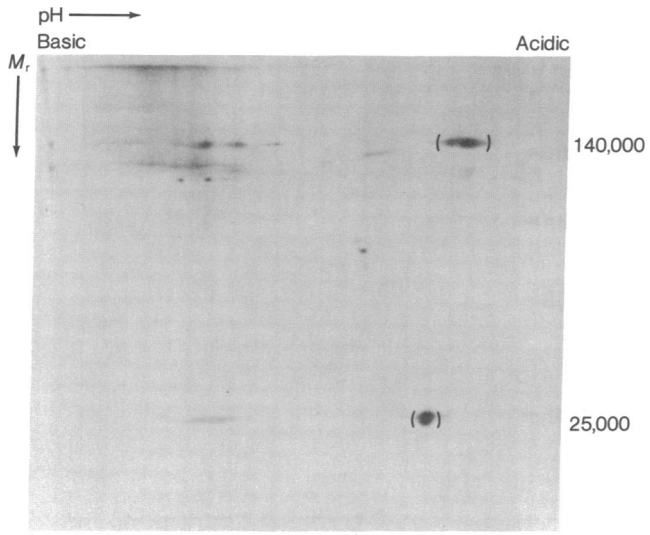

FIG. 3. Pattern of labeled polypeptides obtained by two-dimensional gel electrophoresis of a sample of cochlear nucleus homogenate in lysis buffer (6). RTGP and the 25,000-dalton polypeptide are enclosed by parentheses. The guinea pig was sacrificed 3 hr after an intracochlear injection of 125 μCi of [^{35}S]methionine. Isoelectric focusing was done in the horizontal dimension and the pH gradient ranged from about 4.5 to 7.0. Separation by molecular weight was done in the vertical dimension by using a 10% polyacrylamide gel.

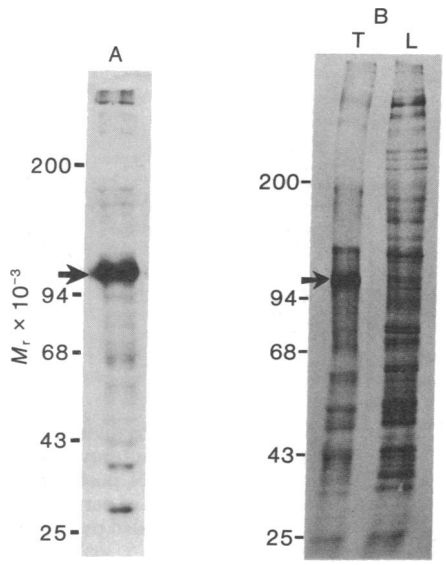

FIG. 4. (*A*) The fucosylated polypeptides which appeared in the cochlear nucleus of a guinea pig sacrificed 2 hr after intracochlear perfusion of 100 μCi of [^{3}H]fucose. The arrow indicates the position of the rapidly turned-over polypeptide. Note the absence of detectable radioactivity in the region of 25,000 M_r. The cochlear nucleus was homogenized in BUST and a sample was applied onto a 4–17.5% polyacrylamide gradient gel for electrophoresis. The apparent molecular weight of the rapidly turned-over protein in this gel is lower than it is in the gel in Fig. 2 possibly because of the presence of 8 M urea in the BUST solubilization buffer used in this experiment (see text). (*B*) Comparison of the labeled polypeptides transported into the cochlear nucleus (T) with those synthesized locally (L) by the glia and neuronal soma in the cochlear nucleus. The arrow indicates the position of RTPG. To label axonally transported proteins, 50 μl of artificial perilymph containing [^{3}H]lysine and [^{3}H]proline (1:1) at 20 μCi/μl was perfused through one cochlea of a guinea pig and the animal was sacrificed 4 hr later. Locally synthesized proteins were labeled in another guinea pig by injecting 2 μl of artificial perilymph containing [^{3}H]lysine and [^{2}H]proline (1:1) at 50 μCi/μl directly into one cochlear nucleus and sacrificing the animal 5 hr later. Each cochlear nucleus was analyzed by electrophoresis in adjacent wells of one gel as described for *A*.

injection of [^{3}H]methionine.§ In all cases, silver grains were localized over primary auditory terminals and neither cell bodies nor glia had any appreciable radioactivity. This result indicated that none of the labeled proteins appearing in the cochlear nucleus were synthesized by glia or neuronal cell bodies within that nucleus.

To substantiate this observation biochemically, we examined the overall population of proteins synthesized by glial and neuronal cells in the cochlear nucleus and compared them with the axonally transported proteins. Although, in the previous experiments, the axonally transported proteins were labeled with [^{35}S]methionine because of its high specific activity and efficiency of detection, for this experiment we felt that it would be better to use a 1:1 mixture of [^{3}H]lysine and [^{3}H]proline because those two amino acids are present in most proteins at a greater frequency than is methionine. In one guinea pig, the [^{3}H]lysine/[^{3}H]proline mixture was injected directly into the cochlear nucleus to label the glial and neuronal cell body proteins and, in another guinea pig, the precursor mixture was perfused through the cochlea as previously described to label the axonally transported proteins. When the patterns of labeled proteins obtained by these two procedures were compared, as shown in Fig. 4*B*, they were found to be quite different. Thus, the labeled proteins that appeared in the cochlear nucleus after exposure of the spiral ganglion to labeled precursor must have been axonally transported. In particular, it was clear that RTGP was not locally synthesized within the cochlear nucleus. This was not the case for the 25,000-dalton polypeptide, because the gel shown in Fig. 4 did not resolve the lower molecular weight bands very well. However, this point was resolved in the final control experiment described below.

To eliminate any remaining doubts that the polypeptides shown in Fig. 2 were axonally transported, the pattern of labeled polypeptides in the eighth nerve itself was analyzed 1 hr after perfusion of [^{35}S]methionine through the cochlea. In Fig. 5, it can be seen that both RTGP and the 25,000-dalton polypeptide were present in the intact eighth nerve, but they were not as prominent there as in the terminals within the cochlear nucleus because they were passing quickly through the nerve and not accumulating. When the transported proteins were caused to accumulate by cutting the nerve, then the relative amounts of labeled transported polypeptides were more similar to the relative amounts seen in the terminals within the cochlear nucleus. The results of these control experiments clearly demonstrated that the labeled polypeptides found in the cochlear nucleus after perfusion of labeled precursor through the cochlea were not products of local synthesis in the cellular elements within the nucleus, but must have been synthesized in the spiral ganglion cells of the organ of Corti and then transported rapidly within the spiral ganglion cell axons of the eighth nerve.

Is RTGP found in neurons other than the spiral ganglion cells? A comparison was made between the auditory system fast-component proteins and those of the visual system to determine if the latter included proteins similar to RTGP and the 25,000-dalton protein as well. The visual system fast component was labeled by giving one guinea pig an intravitreal injection of [^{35}S]methionine. Approximately 3 hr later, the superior colliculus was removed and the labeled proteins present were analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis concurrently with a sample of the cochlear nucleus containing labeled auditory system fast component. The fluo-

§ [^{3}H]Methionine was substituted for the ^{35}S-labeled form because the higher-energy ^{35}S β particle resulted in too much scatter and made it difficult to determine the location of the labeled proteins.

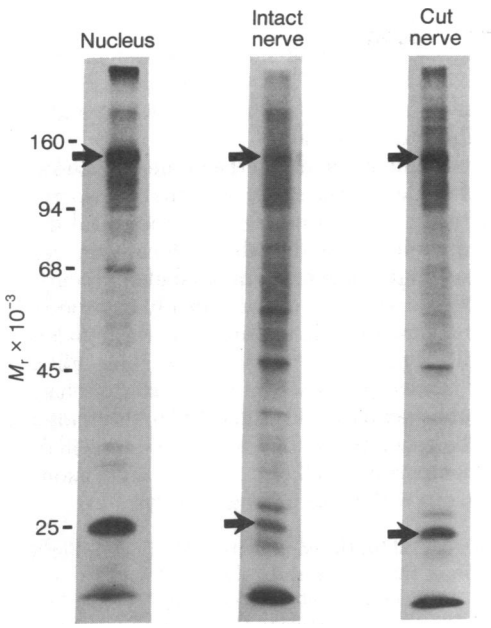

FIG. 5. Comparison of the axonally transported polypeptides labeled with [^{35}S]methionine in the cochlear nucleus, the intact auditory nerve, and the proximal segment of the severed auditory nerve. The arrows mark the positions of RTGP and the 25,000-dalton polypeptide. To label the axonally transported proteins, the cochleas of three guinea pigs were perfused with [^{35}S]methionine. One hour later, the cochlear nucleus and intact auditory nerve were removed from two of the animals. In the third animal, just before the cochlea was perfused, the auditory nerve was severed at the point where it enters the brainstem. The proximal segment of the severed nerve in this animal was removed 2 hr after perfusion. All the tissue samples were homogenized in Laemmli buffer (5) and portions were analyzed by electrophoresis in adjacent wells of a 10% polyacrylamide gel.

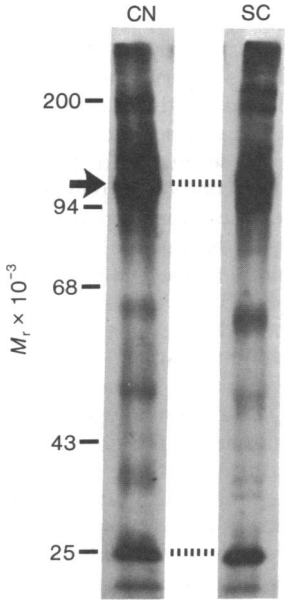

FIG. 6. The major [^{35}S]methionine-labeled polypeptides of the fast component in the spiral ganglion cell terminals in the cochlear nucleus (CN) are compared with those in the retinal ganglion cell terminals in the superior colliculus (SC). The arrow indicates the position of RTGP and the broken lines point out the correspondence between bands in the CN sample representing RTGP and the 25,000-dalton polypeptide and similar bands in the SC sample. The auditory system fast-component polypeptides were labeled as described in *Methods* and the cochlear nucleus was removed 3 hr after perfusion. The visual system fast-component polypeptides were labeled by giving a guinea pig an intravitreal injection of 500 μCi of [^{35}S]methionine in 10 μl of distilled water and the superior colliculus was removed 3 hr later. Both tissue samples were homogenized in BUST and portions were placed in adjacent wells of a 4–17.5% polyacrylamide gradient gel for electrophoretic analysis.

rograph in Fig. 6 suggests that labeled polypeptides with apparent molecular weights similar to those of RTGP and the 25,000-dalton polypeptide of the auditory system are constituents of the visual system fast component. In addition, the two visual system polypeptides had isoelectric points similar to their auditory system counterparts and, as in the auditory system, the 25,000-dalton polypeptide was not fucosylated (data not shown).

DISCUSSION

The experiments in this report illustrate some of the properties of fast axonal transport in a previously unexplored model system, the spiral ganglion cells of the guinea pig. One important result of our initial characterization of this system is the discovery of RTGP. The fact that this polypeptide can be detected in the spiral ganglion cell terminals within 1 hr after the injection of labeled precursor implies that it must be synthesized rapidly to a high specific activity and quickly transported. Actually, both RTGP and the 25,000-dalton polypeptide probably begin to accumulate in the cochlear nucleus even earlier than 1 hr after injection, because we have found trichloroacetic acid-precipitable radioactivity there within as little as 30 min after the injection. Unfortunately, the amount of radioactivity present at that time has been insufficient for analysis by sodium dodecyl sulfate gel electrophoresis. Nonetheless, it is clear that RTGP is transported in the fast component. If one assumes a minimum synthesis time of about 15 min (14), then RTGP moves through the 3-mm length of the auditory nerve in 15 min or at a rate of about 290 mm/day. Because of the shortness of the axons and the fact that we have examined only the rate of accumulation in the terminals, this rate should only be viewed as an approximation. However, it is noteworthy that the rate we have estimated is close to the 240 mm/day reported for the fast component in the mammalian visual system (10).

Our analysis of the auditory system fast component has focused on RTGP and the 25,000-dalton polypeptide because both of these are major constituents of that component and it became apparent early in the study that each had interesting properties. Three different solutions of labeled precursors were employed in the course of this study, [^{35}S]methionine, [^{3}H]-fucose, and a mixture of [^{3}H]lysine and [^{3}H]proline. Consequently, we obtained information about the composition of the two polypeptides in addition to the transport kinetics data. With respect to RTGP, the data showed that it was synthesized in a relatively large amount but was quickly removed from the spiral ganglion cell terminals (Fig. 2). This pattern was observed regardless of the precursor used. Because RTGP was well labeled with all three precursors, we can say that it is composed, in part, of fucose, methionine, and lysine and/or proline. The amounts of each of these precursors in RTGP appear typical of many of the other proteins, judging from the overall profiles of labeled proteins revealed with each precursor. Thus, the data suggest that RTGP is similar to many of the other membrane-associated glycoproteins of the fast component, except for its unusually short life span in the axon terminals. In contrast, the 25,000-dalton polypeptide is not like the other major constituents of the fast component. It is one of the longer-lived fast-component polypeptides, because it is still prominent at 24 hr after injection (Fig. 2) and does not disappear until sometime after 72 hr (unpublished results). Furthermore, it is one of the

few members of the fast component that is not labeled at all with fucose (Fig. 4A) and only poorly labeled with lysine and proline (Fig. 5). These observations suggest that the 25,000-dalton polypeptide is a relatively long-lived methionine-rich constituent of the fast component and that, because it is not fucosylated, it may be serving a function quite different than most of the other fast-component polypeptides.

The comparison of auditory and visual system fast components in Fig. 6 suggests that neither RTGP nor the 25,000-dalton polypeptide is unique to the auditory system. Furthermore, Padilla and Morell (15) have recently reported two rapidly transported polypeptides in the rat geniculostriate pathway with molecular weights similar to those of RTGP and the 25,000-dalton polypeptide. These observations suggest that RTGP and the 25,000-dalton polypeptide or a class of closely related polypeptides are found generally in central nervous system neurons. In spite of these parallels between the fast-component polypeptides of the auditory and visual systems, it is clear that the patterns are not identical (Fig. 6). RTGP is more prominent relative to the other polypeptides in the auditory system than it is in the visual system, and there are other quantitative and qualitative differences between the two profiles as well. A number of investigators have noted the basic similarities between the fast-component proteins in different neurons (16). However, differences have also been reported even between closely related neurons (17). As Black and Lasek (17) have discussed, the basic similarity in the composition of the fast component of different neurons probably reflects the fundamental nature of the fast component, which is the vehicle for the supply of proteins to the agranular reticulum, vesicles, and other membranous structures. The qualitative differences in the fast-component proteins of various neurons are most likely related to the special properties of those neurons that lead to differences in the composition of the structures related to their functions.

One of the clear differences between neurons with different-sized axons is in the amount of material that must be devoted to maintaining the axon. A neuron with a long axon probably requires a greater quantity of certain proteins utilized or deposited within its axon than a neuron with a short axon. Therefore, we suggest that neurons with long axons must synthesize larger quantities of those transported proteins required for maintenance of the axon than neurons with short axons. The differences in the amount of protein synthesized by each would probably be most apparent in the case of those proteins for which the total pool is relatively small, as is true for most of the proteins of the fast component (18).

The rapid disappearance of RTGP from the terminals in the cochlear nucleus could be a result of one of two processes: local elimination (which includes secretion or any type of transformation) or retrograde transport. There are numerous reports that a considerable proportion of transported glycoproteins are associated with synaptic vesicles (14, 19–21), so one possibility is that RTGP is secreted as part of a vesicle. On the other hand, if RTGP is removed from the terminals by retrograde transport, this might reflect a function as a feedback signal, as has been suggested for nerve growth factor, which is known to be retrogradely transported (2).

The fact that we could detect the rapid disappearance of RTGP (Fig. 2) provides indirect information about the precursor pulse time, that is, the length of time that the radioactive precursor perfused through the cochlea is available for incorporation into proteins synthesized in the spiral ganglion cells. Because RTGP has clearly declined between 3 and 6 hr (Fig. 2), this suggests that the precursor has been cleared from the perilymph in 3 hr or less. Thus, the limit of the kinetic resolution in this system is certainly not more than 3 hr and is probably considerably less than that.

We have demonstrated that the guinea pig auditory system is a useful model for the study of axonal transport in neurons with relatively short axons. Because the spiral ganglion cell bodies are contained within the cochlea, they can be easily exposed to a high concentration of labeled precursor. The resulting specific activities of the axonally transported proteins in the spiral ganglion cells are similar to the high levels that we have obtained previously in retinal ganglion cells after an injection of labeled precursor into the posterior chamber of the eye (unpublished data). The fact that highly radioactive proteins can be generated in the auditory system will facilitate the detailed comparison of all rate components of axonal transport in short axons with those in long axon systems.

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1. Grafstein, B. (1977) in *Handbook of Physiology: The Nervous System*, eds. Brookhart, J. M. & Mountcastle, V. B. (Am. Physiol. Soc., Washington, DC), Vol. 1, pp. 691–717.
2. Grafstein, B. & Forman, D. (1980) *Physiol. Rev.*, in press.
3. Schubert, P., Lux, H. D. & Kreutzberg, G. W. (1971) *Acta Neuropathol. Suppl.* **5**, 179–186.
4. Konishi, T. & Kelsey, E. (1973) *Acta Oto-Laryngol.* **76**, 410–418.
5. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
6. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021.
7. Ames, G. F.-L. & Nikaido, K. (1976) *Biochemistry* **15**, 616–623.
8. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.
9. Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56**, 335–341.
10. Willard, M., Cowan, M. X. & Vagelos, P. R. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2183–2187.
11. Black, M. M. & Lasek, R. J. (1979) *Brain Res.* **171**, 401–413.
12. Pitt-Rivers, R. & Impiombato, F. S. A. (1968) *Biochem. J.* **109**, 825–830.
13. Weber, K. & Osborn, M. (1975) in *The Proteins*, eds. Neurath, H. & Hill, R. L. (Academic, New York), Vol. 1, pp. 179–223.
14. Droz, B., Koenig, H. L. & DiGiamberardino, L. (1973) *Brain Res.* **60**, 93–127.
15. Padilla, S. S. & Morell, P. (1979) *Trans. Am. Soc. Neurochem.* **10**, 207 (abstr.).
16. Wilson, D. L. & Stone, G. C. (1979) *Annu. Rev. Biophys. Bioeng.* **8**, 27–45.
17. Black, M. M. & Lasek, R. J. (1978) *J. Neurobiol.* **9**, 433–443.
18. Stone, G. C., Wilson, D. L. & Hall, M. E. (1978) *Brain Res.* **144**, 287–302.
19. DiGiamberardino, L., Bennett, G., Koenig, H. L. & Droz, B. (1973) *Brain Res.* **60**, 147–159.
20. Schwartz, J. H. (1979) *Trans. Am. Soc. Neurochem.* **10**, 145 (abstr.).
21. Weiss, D. G., Krygier-Brévart, V., Mehl, E. & Kreutzberg, G. W. (1979) *Biol. Cellulaire* **34**, 59–64.