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REUTILIZATION OF PRECURSOR FOLLOWING AXONAL TRANSPORT OF [^{3}H]PROLINE-LABELED PROTEIN

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SUMMARY

In further studies on axonally transported protein in the goldfish visual system, the turnover of rapidly transported [^{3}H]proline-labeled protein was examined. It was found that: (1) a fraction of the rapidly transported protein has a relatively short half-life; (2) [^{3}H]proline released following proteolysis of transported protein is efficiently reutilized for tectal protein synthesis, as inferred from an increased labeling of nuclear protein in the contralateral tectum (COT) relative to that in the ipsilateral tectum (IOT); (3) a small amount of [^{3}H]proline arrives in the COT by axonal flow of the free amino acid; and (4) [^{3}H]leucine and [^{3}H]asparagine are less efficiently reutilized than [^{3}H]proline.

These findings may relate to the phenomenon of transneuronal transfer of radioactivity which has been observed with [^{3}H]proline as precursor. The extensive reutilization of [^{3}H]proline may account for part or all of the labeling at secondary synaptic sites. The results suggest that asparagine may be highly suitable for radioautographic identification of primary neuronal fields.

INTRODUCTION

The use of labeled axonally transported proteins to trace neuroanatomical pathways radioautographically is based on the known accumulation of the rapidly transported material in presynaptic regions of axons whose cell bodies were near the site of labeled amino acid precursor injection[5,18,19,28]. [^{3}H]Proline is a well-suited precursor for many systems[6,25]. It has been noted in a number of studies with this precursor that the synaptic beds of secondary fields are also significantly labeled[4,8,12,30]. This has led to the suggestion that intact proteins may be transferred transneuronally[12,13,26]. The present studies were undertaken to establish whether the radioautographic findings could instead be explained by incorporation into protein

of neighboring neuronal perikarya of free [^{3}H]proline that migrated axonally or that was released upon proteolysis of transported protein.

MATERIALS AND METHODS

Goldfish *(Carassius auratus)*, 6–7 cm in length, were obtained from Ozark Fisheries (Stoutland, Mo.) and maintained at 20 ± 1 °C. The following radiolabeled amino acids were obtained from New England Nuclear (Boston, Mass.): L-[2,3-^{3}H]-proline (30 Ci/mmole), L-[4,5-^{3}H]leucine (40 Ci/mmole), and L-[G-^{3}H]4-hydroxy-proline (4.4 Ci/mmole). L-[2,3-^{3}H]Asparagine (12–13 Ci/mmole) was purchased from Schwarz/Mann (Orangeburg, N.J.). Cycloheximide (Actidione, CXM) was obtained from ICN (Cleveland, Ohio) and acetoxycycloheximide (AXM) was a gift of Dr. T. C. McBride, Chas. Pfizer and Co. (Maywood, N.J.).

Radioactive solutions were evaporated to dryness under nitrogen and redissolved in distilled water immediately prior to use. For intraocular (IO) injections, 5 μl were delivered into the vitreous by means of a 50 μl Hamilton syringe and a 30-gauge needle fitted with a polyethylene sleeve to limit penetration to 2 mm. Intracranial injections were routinely in 10 μl of 0.15 *M* NaCl.

Determination of radioactivity

Samples of optic tecta or whole brain which were not to be subjected to subcellular fractionation were frozen on dry ice immediately after removal. The tissue was then homogenized with a glass-to-glass homogenizer in a small volume of cold distilled water. Radioactivity in acid-soluble and -insoluble fractions was determined by a trichloroacetic acid precipitation filter paper method[22,24]. When L-[G-^{3}H]4-hydroxyproline transport was examined, radioactivity on dried filters was measured without an acid wash, since acid-insoluble radioactivity was absent. Goldfish eyes were homogenized with a Brinkmann Polytron Type PT-1020.

Preparation of purified nuclei

Nuclei were purified according to the method of Løvtrup-Rein and McEwen[20]. Left and right tecta from 35 to 50 goldfish were rinsed separately in ice-cold saline, then homogenized with a loose fitting Teflon-glass homogenizer in 10 vol. of 0.32 *M* sucrose, 1 m*M* in sodium phosphate buffer (pH 6.5), and containing 3 m*M* $MgCl_2$ and 0.25% Triton X-100. After centrifugation at 1000 × *g* for 7 min, the pellet was washed twice in homogenization medium without detergent and purified by centrifugation through 2.1 *M* sucrose. Purity of the nuclear fraction was examined by light microscopy following staining with cresyl violet. Aliquots of the resuspended nuclear pellets were analyzed for radioactivity as described above. Protein concentration in nuclei and homogenates was determined spectrophotometrically[21].

To examine possible contamination of nuclei with labeled non-nuclear protein, a crude nuclear pellet (1000 × *g* precipitate) from the brains of 20 goldfish was rehomogenized with a 1000 × *g* soluble protein fraction from the brains of 20 fish previously labeled for 24 or 48 h by intracranial administration of 3 μCi of [^{3}H]proline

TABLE I

Time course of labeling of tectal nuclear protein after IO injection of [^{3}H]proline

Goldfish were injected intraocularly with 8 μCi [^{3}H]proline. Tecta were removed from groups of 50 fish at the indicated times, nuclei were purified, and the specific radioactivity of nuclear protein and tectal homogenate protein was determined.

	Time after IO injection (h)				
	3	*6*	*12*	*24*	*48*
	Specific radioactivity (disint./μg protein)				
Homogenate					
Contralateral	2.02	36.85	46.0	48.8	47.7
Ipsilateral	0.53	0.41	2.03	3.04	1.78
Nuclei					
Contralateral	0.22	4.56	7.94	10.37	13.23
Ipsilateral	0.24	0.26	0.53	0.83	0.58
COT–IOT $\frac{\text{Nuclei}}{\text{Homogenate}} \times 100$ (%)	0	11.8	16.8	20.8	27.7

per fish. After reserving an aliquot of the rehomogenized mixture for specific activity determination, purified nuclei were prepared.

Paper chromatography of hydroxyproline

The acid-soluble fraction of a tectal homogenate was extracted with ether, lyophilized to dryness, redissolved in a small volume of distilled water, applied to a strip of Whatman No. 1 filter paper and subject to descending chromatography in *n*-butanol–acetic acid–water (3:1:1) or *n*-propanol–1 *N* NH_4OH (7:3). The position of hydroxyproline standard in a duplicate strip was detected with ninhydrin. The sample strip was cut into 2 cm sections and combusted in a Packard sample oxidizer prior to liquid scintillation spectrometry.

RESULTS

Labeling of tectal nuclear protein

The possible local incorporation of transported proline into tectal protein was investigated by examining the labeling of purified tectal nuclei obtained at various times after intraocular injection of [^{3}H]proline. Since cell nuclei are not axonally transported, increased radioactivity in nuclear protein in the contralateral optic tectum (COT) relative to that in the ipsilateral optic tectum (IOT) would imply utilization of axonally transported precursor (whether as free amino acid or by degradation of labeled protein) for tectal synthesis of nuclear protein.

Goldfish were injected into the right eye with [^{3}H]proline and nuclei were purified from both tecta at 3, 6, 12, 24 and 48 h after injection (Table I). A COT–IOT

difference in nuclear protein labeling is apparent at 6 h and continues to increase up to at least 48 h. In contrast, the homogenate specific activity remains constant during the 12–48 h interval. The observed increased labeling of nuclear protein COT relative to IOT could result from: (a) tectal incorporation of [^{3}H]proline supplied by axonal transport of free proline and/or by breakdown of transported [^{3}H]proline-labeled protein and reutilization of precursor; or (b) contamination of the purified nuclei with highly radioactive transported protein. The latter possibility was examined in a mixing experiment, detailed in Methods, in which an unlabeled crude nuclear preparation from goldfish brain was rehomogenized together with a [^{3}H]proline-labeled postnuclear supernatant fraction. The specific radioactivity of the purified nuclei obtained was 9.74% ± 1.04 (mean ± S.E., n = 5) of the mixed homogenate specific activity. Comparison of this percentage with those shown in Table I indicates that contamination of nuclei with non-nuclear protein can account for a significant fraction of nuclear radioactivity at earlier times after IO injection, but not for the increase seen at later times, which at 24 h is twice that expected were contamination responsible for nuclear protein labeling.

CXM inhibition of nuclear labeling

Since contamination of nuclei can account at most for only a fraction of the observed nuclear protein radioactivity in the contralateral optic tectum, we next explored the possibility that there was local incorporation of transported labeled precursor. This was confirmed by examining the effect of inhibition of tectal protein synthesis on nuclear protein labeling. Goldfish were injected intracranially (IC) with 10 μg of cycloheximide (CXM) at 6 h intervals beginning 12 h after IO administration of [^{3}H]proline. From previous studies[17], this dosage schedule of CXM is known to inhibit tectal protein synthesis by at least 75–85% throughout the 12–40 h period. Control fish received IC injections of saline. Protein radioactivity in whole tectal homogenate and the nuclear fraction, as well as in the eye, was determined after 40 h (Table II). CXM treatment had no significant effect on incorporation of [^{3}H]proline into protein in the eye or into ipsilateral tectal homogenate and nuclear protein. This result indicates that incorporation of injected precursor was complete before the CXM was administered and that systemically labeled tectal protein does not turn over significantly during the period of protein synthesis inhibition. In contrast, and in agreement with a similar recent study[17], the specific radioactivity of homogenate protein in the contralateral tectum was decreased by 25% relative to saline-treated control fish. Nuclear protein labeling in the contralateral tectum was depressed 37% in the presence of CXM. Comparison of the data in Table II with that in Table I shows that the increase in nuclear protein specific radioactivity which was expected to occur between 12 and 40 h was completely prevented by CXM treatment. Although in this experiment, tectal radioactivity was not examined at the onset of the CXM treatment (12 h), homogenate specific activity in all fish at 12 h should have been equal to that of the saline-treated fish at 40 h. The nuclear protein labeling (COT) in both CXM and saline-treated groups at 12 h would then have been at about 17% that of the homogenate (Table I), a value similar to that obtained in the CXM-treated fish at 40 h.

TABLE II

Effect of intracranial administration of cycloheximide on tectal protein radioactivity following intraocular injection of [^{3}H]proline

Goldfish were injected IC with 10 μg CXM in 10 μl saline or with 10 μl saline (control) at 12, 18, 24, 30 and 36 h after injection of 8 μCi of [^{3}H]proline. The CXM treated and control fish were each divided into two groups of 45. Tecta and eyes were removed at 40 h after IO injection. Tectal nuclei were prepared and radioactivity was determined. Data for both control groups (A,B) and both CXM treated groups (C,D) are presented.

	Specific radioactivity (disint./min/μg protein)			
	Control		*CXM*	
	A	*B*	*C*	*D*
Homogenate				
Contralateral	75.7	77.3	60.6	54.9
Ipsilateral	1.09	0.92	1.06	0.96
Eye	1758	1619	1302	1813
Nuclei				
Contralateral	20.5	17.3	11.7	12.9
Ipsilateral	0.65	0.59	0.59	0.65
COT–IOT Nuclei / Homogenate $\times$ 100 (%)	26.6	21.9	14.9*	16.1*

* CXM treated nuclei as a percentage of average control homogenate.

These data support the conclusion that local incorporation of [^{3}H]proline into tectal protein is responsible for much of the observed increment in nuclear protein specific radioactivity in the contralateral tectum which occurs after the arrival of the peak of axonally transported protein. The decreased radioactivity in contralateral homogenate protein in the presence of CXM suggests that a substantial fraction of the rapidly transported protein may have a relatively short half-life. The breakdown of these proteins could then serve as a source of labeled amino acid for reincorporation into tectal protein. The results, however, do not preclude the additional possibility that some free proline may be transported from the eye *via* the optic nerve to the tectum where it could be utilized for local protein synthesis.

Axonal flow of free proline

In order to demonstrate the axonal flow of a free amino acid, it is necessary to show that the arrival of acid-soluble radioactivity in the nerve ending region is not dependent upon the transport of labeled protein. This was investigated by determining the effect of inhibition of retinal protein synthesis on the arrival of protein-bound and acid-soluble radioactivity in the optic tectum following IO injection of [^{3}H]-proline. Goldfish were injected IO with the potent glutarimide protein synthesis inhibitor, acetoxycycloheximide (AXM) or saline 30 min prior to IO administration

TABLE III

Effect of inhibition of retinal protein synthesis on the appearance of acid-soluble radioactivity in the optic tectum following IO injection of [^{3}H]proline

Goldfish were injected IO with 5 μl saline or 5 μl of 0.01 μg AXM/μl 30 min prior to IO administration of 8 μCi [^{3}H]proline. Acid-soluble and insoluble tectal radioactivity was determined in groups of 6 fish at 8 h (A) or 24 h (B) after [^{3}H]proline injection. Data are expressed as the difference in specific radioactivity between contralateral and ipsilateral optic tecta.

	Specific radioactivity (disint./min/μg protein)			
	COT–IOT/protein	*(% inhibition)*	*COT–IOT/acid soluble*	*(% inhibition)*
A. Control	39.6	(98.8)	0.78	(74.7)
AXM	0.45		0.20	
B. Control	53.4	(96.0)	1.13	(67.1)
AXM	2.1		0.37	

of [^{3}H]proline. Tecta were removed at 8 or 24 h and acid-soluble and -insoluble radioactivities were determined (Table III). In agreement with previous studies[10,24], both saline-treated groups showed a COT–IOT difference in acid-soluble radioactivity which was about 2% that of the protein-bound radioactivity. Also, as expected[23], inhibition of retinal protein synthesis by AXM essentially blocked the appearance of rapidly transported [^{3}H]proline-labeled protein. The arrival of acid-soluble radioactivity was, however, only partially inhibited by AXM treatment, suggesting the axonal flow of the labeled precursor. This conclusion is based on the assumption that in each measurement, essentially all of the acid-soluble radioactivity is in proline. Paper chromatography revealed that this was not the case. After a 24 h pulse, proline represented 41% of the acid-soluble radioactivity in the control COT and 21% in the AXM-treated COT. In each group there was still a COT–IOT difference in [^{3}H]-proline, but AXM treatment caused an 88% inhibition, to be compared with the 96% inhibition of the arrival of transported protein-bound [^{3}H]proline. The data thus do not unequivocally demonstrate the axonal flow of free [^{3}H]proline.

Another possible approach to the detection of axonal flow of free amino acid is the use of an amino acid analog which is not incorporated into protein. A proline analog, L-4-hydroxyproline, was a suitable choice for this study since it competes with proline for active transport in vertebrate tissues[11], it is not incorporated into protein[27], and it might share with proline its advantage for axonal transport studies, i.e., a poor uptake from blood into brain. Tectal radioactivity was examined at various times after IO injection of [^{3}H]hydroxyproline (Fig. 1). A COT–IOT difference appeared at 3–6 h, peaked at 12 h and began to fall off by 24 h. Recovered radioactivity in both tecta behaved chromatographically as hydroxyproline.

Arrival of transported [^{3}H]hydroxyproline was inhibited by 73% if injected together with 4.5 μmoles L-proline. This dose of proline is 500 times the estimated free proline pool size in the goldfish eye[24]. A 4500-fold dilution of the [^{3}H]hydroxy-proline specific radioactivity by the addition of 4.5 μmoles of unlabeled L-hydroxy-proline caused only 87% inhibition. This suggests that the uptake of hydroxyproline

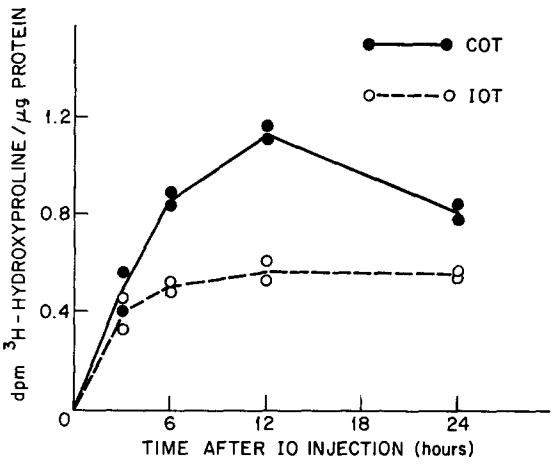

Fig. 1. Axonal transport of [^{3}H]hydroxyproline. Goldfish were injected into the right eye with 4 μCi of [^{3}H]hydroxyproline. Tecta were removed at 3, 6, 12, 24 and 48 h. Specific radioactivity in contralateral and ipsilateral tecta was determined in duplicate groups of 7–8 fish.

into retinal ganglion cells, or its axonal transport is saturable only at very high concentrations. Intraocular injection of 4.5 μmoles of D-allo-hydroxyproline or IO treatment with AXM had no effect on the arrival of [^{3}H]hydroxyproline in the contralateral tectum. IO injection of 2 μg of colchicine 24 h prior to administration of [^{3}H]-hydroxyproline (12 h pulse) resulted in a 53% inhibition of the accumulation of [^{3}H]-hydroxyproline in the contralateral tectum. The same colchicine treatment caused a 58% inhibition of the arrival of transported [^{3}H]proline-labeled protein in the tectum.

The percent of the dose of hydroxyproline transported ranged from 0.0005 to 0.003. It can be inferred that free proline is also axonally transported, but a quantitative estimate cannot be made. The lifetime of the two amino acids in the eye, their relative uptake into retinal ganglion cells and their disposition in the retina and in the tectum are all factors which would affect the appearance of free amino acid in the tectum. Taken together, these experiments indicate that there is axonal flow of free proline in the goldfish visual system, but that it makes only a minor contribution to the supply of free proline for local incorporation into protein of the contralateral tectum compared to that from breakdown of rapidly transported protein.

Incorporation of transported [^{3}H]leucine and [^{3}H]asparagine into tectal protein

In a previous long-term study of the turnover of axonally-transported protein in the goldfish visual system, it was found that the half-life of slowly transported proline-labeled protein following IO injection was 103 days while that of leucine-labeled protein was 67 days, when measured over the interval between 63 and 228 days[24]. This difference suggested that proline was reutilized for tectal protein synthesis more efficiently than leucine. Since labeling of tectal nuclear protein by [^{3}H]proline in the present study also indicated that significant reutilization was occurring, the possibility that [^{3}H]leucine would be incorporated less into nuclear protein was

TABLE IV

Comparison of labeling of tectal nuclear protein after IO injection of [^{3}H]proline, [^{3}H]asparagine or [^{3}H]leucine

Goldfish, in groups of 35–50 were injected with either 8 μCi [^{3}H]proline, 5.8 μCi [^{3}H]asparagine or 20 μCi [^{3}H]leucine. Tecta were removed at 24 or 48 h. Acid-soluble and -insoluble homogenate radioactivity and protein-bound nuclear radioactivity were determined. The COT/IOT ratio and the % acid-soluble values are averages of 2–5 determinations. The [^{3}H]proline nuclear labeling values are from single measurements. Those for [^{3}H]asparagine and [^{3}H]leucine are each averages of 2–3 determinations.

		Specific radioactivity (disint./min/μg protein)		
		Proline	*Asparagine*	*Leucine*
COT/IOT Acid-insoluble		21.3	15.4	1.62
COT/IOT Acid-soluble / Acid-insoluble	100 (%)	2.04	0.95	0
COT/IOT Nuclei / Homogenate	100 (%) 24 h	20.8	12.7	0.96
	48 h	27.7	15.1	3.4

examined. Similar experiments were also carried out with [^{3}H]asparagine, which, like proline, is a good precursor of axonally transported protein.

In addition to the degree of labeling of nuclear protein, the presence of a COT–IOT difference in acid-soluble radioactivity was examined. The results presented in Table IV compare the data obtained with [^{3}H]leucine and [^{3}H]asparagine to that already presented for [^{3}H]proline. While both proline and asparagine have a similarly high COT/IOT ratio of acid-insoluble radioactivity, asparagine is less efficient than proline in labeling tectal nuclear protein. Asparagine also shows about half as much COT–IOT acid-soluble radioactivity as proline. In contrast, transported [^{3}H]leucine is very inefficient in labeling tectal protein and shows no COT–IOT difference in acid-soluble radioactivity. Because the COT/IOT ratio with [^{3}H]leucine is so low, a COT–IOT difference in acid-soluble radioactivity at the 1–2% level may not have been detectable. However, the presence of a 10–20% COT–IOT difference in nuclear protein labeling should have been demonstrable.

DISCUSSION

When an isotopic amino acid precursor is injected into the goldfish eye, radioactive protein is subsequently found in both the contralateral and ipsilateral visual tectum[23]. Since the visual pathways are believed to be completely crossed, the difference between the radioactivity in contralateral and ipsilateral tectal protein is a convenient indication of the amount of protein that had been transported axonally[23],

while the ratio of the two tectal radioactivities is a measure of the efficacy of the injected isotope in selectively labeling axonally transported protein[10]. In comparing 18 tritium-labeled amino acids, we previously noted that proline and asparagine gave particularly high ratios[10]. The axonal transport of labeled protein is the basis for a radioautographic technique for tracing neural pathways[5,18,19,28]. Our study suggested that leucine, a popularly used precursor, was in this case much less desirable than proline and asparagine. We subsequently demonstrated the radioautographic use of [^{3}H]proline in the goldfish visual system[25], and it has since been used widely in a number of anatomical sites in different species and found to be a highly useful agent for the radioautographic tracing technique[6].

The fate of the axonally transported protein which accumulates at presynaptic sites is of interest from a number of standpoints. The appearance of labeled protein outside of what is considered to be a primary terminal field is well-established, and has been used to advantage in tracing the terminal fields of second-order neurons[4,8,30]. To explain this, it has been proposed that intact proteins may migrate transneuronally[12], and while there is no question that such a phenomenon would have great significance in regard to trophic effects, etc., the present results suggest another mechanism.

This problem was approached biochemically by examining the labeling of a purified nuclear fraction prepared from tectum. Since this subcellular organelle could not be labeled directly by its axonal transport, it was a convenient marker for protein that could only have been synthesized locally in the tectum. Another such marker which might have been examined was ribosomal protein. However, there is some controversy regarding the presence of RNA in the axon or nerve ending[2,15] so that this fraction might have been a less convincing indicator of labeling outside of the primary terminal field.

The increment in nuclear protein labeling which is observed in the contralateral tectum after IO injection of [^{3}H]proline was shown to result from reutilization of [^{3}H]proline supplied by turnover of the rapidly transported protein. Another possible source of precursor, axonal flow of free proline accounted for only a small fraction of the free proline supplied to the tectum. In general, free amino acids have been considered not to be axonally transported. Earlier studies failed to demonstrate axonal flow of cycloleucine in the goldfish visual system[23] possibly because of high background (IOT) labeling with this amino acid. Recently the axonal transport of [^{3}H]-leucine has been demonstrated in the garfish olfactory nerve[3]. Axonal flow of [^{14}C]-proline has been observed in carp optic nerve but only trace amounts were found in the tectum[7]. Demonstration in the present study of the axonal transport of the proline analog, hydroxyproline, may have been facilitated by the low IOT background attributable to its poor uptake into the brain from the circulation.

The 25% decrease in tectal homogenate specific activity following intracranial administration of cycloheximide (Table II) indicates that a significant fraction of the rapidly transported protein has a relatively short half-life, in agreement with recent studies in the rabbit visual system[16,31]. The [^{3}H]proline released by proteolysis of transported protein may be more available than leucine for local reincorporation, since it is not so rapidly cleared from the brain by the circulation or by breakdown as

is [^{3}H]leucine[24]. This is consistent with the apparent minimal reutilization of [^{3}H]-leucine, as evidenced by its poor labeling of tectal nuclear protein and the previously observed shorter half-life of slowly transported [^{3}H]leucine-labeled protein compared with [^{3}H]proline-labeled protein[24].

A relationship between reutilization of precursor following breakdown of transported labeled protein and the subsequent appearance of radioactivity outside of the primary terminal field is suggested by these studies. The amount of labeling of tectal nuclear protein, which represents only a fraction of the total incorporation into neighboring perikarya, is consistent with the previously observed radioautographic grain density in areas of the goldfish optic tectum not receiving direct input from the retina[17,25]. The decrease in grain density in tectal regions adjacent to the primary terminal field following CXM treatment[17] supports reutilization of precursor as the mechanism for this labeling. From similar experiments in the chick ciliary ganglion[9], Droz et al. concluded that reincorporation of [^{3}H]lysine accounted for at least 90% of the observed labeling in postsynaptic perikarya. Additional support for the reutilization hypothesis comes from radioautographic experiments with different precursors in which superior labeling of secondary neuronal fields with proline was demonstrated. Transneuronal labeling of the monkey visual cortex was observed radioautographically after IO [^{3}H]proline[30], but much less labeling of the cortex above background was found with [^{3}H]leucine. (In the monkey, [^{3}H]leucine does not produce high background labeling[14,29].) Hendrickson has observed radioautographic grain density above background in postsynaptic neurons of the monkey lateral geniculate after IO [^{3}H]leucine[14], but this is evidently not sufficient to result in significant labeling of the visual cortex. The superiority of [^{3}H]proline over [^{3}H]leucine as a precursor of transneuronally labeled protein has also been observed in the mouse visual system[13]. The observed radioautographic and biochemical differences between leucine and proline constitute further evidence against the possibility that transneuronal transport of intact proteins makes a quantitatively significant contribution to the labeling at secondary synaptic sites since the amount of labeled protein transported would not be expected to depend upon the choice of precursor.

While the use of [^{3}H]proline frequently has many advantages for studies of axonal transport and for radioautographic tracing methods, in those situations where it is desirable to minimize the amount of reutilization, [^{3}H]asparagine may prove to be a more suitable precursor. Data are not available on the half-life of [^{3}H]asparagine-labeled goldfish brain protein, but the tectal nuclear protein labeling results suggest that it is reutilized no more than one-third to one-half as much as [^{3}H]proline.

The present observations taken together are consistent with the suggestion that breakdown of transported protein and reutilization of precursor make a substantial contribution to the appearance of labeled protein at secondary synaptic sites, however, they do not preclude the possibility of transneuronal or even transsynaptic migration of proteins. There is some evidence, based on electrophoretic analysis of the proteins, in support of the transfer of labeled protein from nerve to muscle[1]. It may be that the putative transneuronal transfer of intact protein and the processes of breakdown and

reutilization of precursor are interrelated. Although it is believed that the reincorporation of precursor can occur only in perikarya, or perhaps dendrites, the site of degradation of transported protein could be in the preterminal and terminal regions of the axon as well as in the neighboring cell bodies or dendrites. In the former case, amino acid released by proteolysis would have to diffuse locally, be taken up into neighboring cells and reincorporated into protein. In the latter case, some proteins may be transferred intact to neighboring cells, perhaps by endocytosis, followed by degradation of the transferred protein and subsequent reutilization of the released amino acid. The available data are consistent with either mechanism and it is possible that both may be operating.

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