Post-translational Processing and Turnover Kinetics of Presynaptically Targeted Amyloid Precursor Superfamily Proteins in the Central Nervous System*

(Received for publication, November 20, 1997, and in revised form, February 16, 1998)

Alvin W. Lyckman‡‡, Anna Maria Confalonieri‡‡, Gopal Thinakaran†, Sangram S. Siriošia, and Kenneth L. Moya‡‡‡‡

From the ‡‡INSERM U334, Service Hospitalier Frédéric Joliot, Commissariat à l’Energie Atomique, DSV/DRM, Orsay, France, §Laboratorio de Metabolismo e Biocompatica Patologica, Istituto Superiore di Sanità, Rome, Italy, †Neuropathology Laboratory, Johns Hopkins University School of Medicine, Baltimore, Maryland, and **CEA-CNRS URA 2210, Service Hospitalier Frédéric Joliot, DRM/DSV, Orsay, France

The amyloid precursor superfamily is composed of three highly conserved transmembrane glycoproteins, the amyloid precursor protein (APP) and amyloid precursor-like proteins 1 and 2 (APLP1, APLP2), whose functions are unknown. Proteolytic cleavage of APP yields the β4A peptide, the major component of cerebral amyloid in Alzheimer’s disease. Here we show that five post-translationally modified, full-length species of APP and APLP2 (but not APLP1) arrive at the mature presynaptic terminal in the fastest wave of axonal transport and are subsequently rapidly cleared (mean half-life of 3.5 h). Rapid turnover of presynaptic APP and APLP2 occurs independently of visual activity. Turnover of the most rapidly arriving APP species was accompanied by a delayed accumulation of a 120-kDa, APP fragment lacking the C terminus, consistent with presynaptic APP turnover via constitutive proteolysis. Turnover of APLP2 was not accompanied by detectable APLP2 fragment peptides, suggesting either that APLP2 either is more rapidly degraded than is APP or is retrogradely transported shortly after reaching the terminus. A single 150-kDa APLP2 species containing the Kunitz protease inhibitor domain is the major amyloid precursor superfamily protein transported to the presynapse. Presynaptic APP and APLP2 are sialylated and of cerebral vessels and senile plaques (1). Interest in the regulation of APP expression arose from it being recognized as the parent protein of β4A, the major peptide constituent of cerebral vascular and senile plaque amyloid (2). APP is one of the three proteins encoded by genes in the mammalian amyloid precursor superfamily (APSF; see Fig. 1A), which also has two genes encoding APLP1 and APLP2 (3). Although the APP gene is the only APSF gene that encodes β4A, the conservation of gene structure (4) and domain homology (5) among the APSF proteins suggests that they may share significant overlap in function and expression, and thus, that amyloidogenic processing of APP may be influenced in parallel with processing of APLP1 and/or APLP2. For instance, APPS proteins from various phyla share highly conserved extracellular zinc and heparin binding domains (3), single membrane-spanning domains (4, 6), and intracellular phosphorylation sites (7). Additionally, APP and APLP2 genes both contain N-linked glycosylation sites (8), splice-specific chondroitin sulfate glycosaminoglycan (CSGAG) attachment sites (9–11), and, alternatively, spliced exons that encode the Kunitz protease inhibitor (KPI) domain (4, 12). Various combinations of these modifications yield several isoforms for APSF proteins that could differ significantly in terms of metabolism, transport and protein function. To better understand the regulation of neurona1ly expressed APSF proteins in vivo, we quantitatively and biochemically examined APPS protein expression and turnover in a major pathway of the central nervous system, the primary visual projection (Fig. 1B). Previous studies have indicated that APP is synthesized by neuronal populations and that it undergoes fast anterograde transport (13–16), although the fate of axonally transported APP is relatively understudied, and no studies have examined APLP1 or APLP2 for axonal transport. Inspection of translated sequences reveals that all three APSF proteins carry a consensus cholinergic binding region in the cytoplasmic tail. Various published data allude to a relationship between APP metabolism and endocytotic/endosomal membrane recycling (17, 18). Considering the tight regulation of the exocytotic-endocytotic cycle during excitation-release coupling in the presynaptic terminal (19), it was hypothesized that metabolism of APP (and possibly of APLP1 and/or APLP2) would...
be coupled to clathrin-mediated endocytosis driven by activity-dependent presynaptic release of neurotransmitter. As presynaptic turnover would be suggestive of proteolysis, we also examined presynaptically targeted APSF proteins for evidence of proteolytic processing. Finally, the extracellular domains of APP and APLP2 may carry glycosylations (8) that could potentially interact with extracellular materials (20, 21) or cell membranes (22) at the synapse. Thus, we examined neuronally expressed, presynaptically targeted APSF proteins for various types of glycosylation. In summary, we used a model neuron of the central nervous system to document the neosynthesis, multiple glycosylation, and rapid axonal transport of five distinct APP and APLP2 species to presynaptic terminals in vivo. We show, moreover, that these APP and APLP2 variants undergo rapid turnover in the presynaptic terminus independent of presynaptic neuronal activity.

**EXPERIMENTAL PROCEDURES**

(35S)Met/Cys-labeling of Presynaptic Proteins—Adult Syrian hamsters (—150 g) were anesthetized with a xylazine/Ketalar mixture (ratio 1:9; 0.3 µg/kg body weight). One eye per hamster was intraocularly injected with 100 µl of [35S]Met (—1100 Ci/mmol, Expev255-26, NEN Life Science Products) in 2 ml of normal saline. This technique allows radiolabeling of nascent protein in retinal ganglion cells (RGCs) whose axons innervate distinct target regions in the central nervous system (Fig. 1B). Animals were sacrificed at various intervals after radiolabeling by pentobarbital overdose, after which the superior colliculus (SuC) and lateral geniculate nuclei (LGN) contralateral to the injected eye were rapidly dissected and frozen at —70 °C.

Separation of Labeled Proteins—Each dissected SuC was individually homogenized in a glass-glass tissue homogenizer in 550 µl of lysis buffer containing 9.5 mM urea, 2% w/v Nonidet P-40, 5% v/v 2-mercapto ethanol, and 6% v/v ampholines (pH 3.5–5.0, 5.0–8.0, 3.5–10.0) (Amersham Pharmacia Biotech) in the ratio 5:7:3. 500 µl of the homogenate was submitted to isoelectric focusing. Proteins were separated in the molecular weight dimension by polyacrylamide gel (5–15% linear gradient) electrophoresis in the presence of SDS in Laemmlı buffer. Proteins were then transferred to PVDF membranes (Immobilon-P; Millipore) in SDS-free transfer buffer (25 mM Tris, 192 mM glycine). We then transferred to PVD membranes (Immobilon-P; Millipore) in SDS-free transfer buffer (25 mM Tris, 192 mM glycine).

Identification of Labeled Proteins—After PVDF transfers had been used to generate storage phosphor images (see below), the same transfers were placed against, autoradiography film (Biomat, Eastman Kodak Co.) for 5–7 wk, after which the same PVDF transfers were processed for immunoblotting using enhanced chemiluminescence (Kodak Co.) for 5–7 wk, after which the same PVDF transfers were placed against autoradiographic film (Biomax, Eastman Kodak Co.) in 2 m Crime. We then compared the autoradiogram with the storage phosphor image. Identified spots on the immunoblots that did not overlap with spots on the autoradiograms were considered not relevant to our study of transported protein. Such spots may result from low expression of APSF proteins in retinal target tissues.

Quantitative Analysis of Labeled Protein—PVDF membranes were placed on storage phosphor screens for 7–12 days. Storage phosphor screens were read on a PhosphorImager (Molecular Dynamics). The digitized phosphor signals were quantitated with IPLab Gel software (IPLabs, Vienna, VA), and data were statistically analyzed using Excel (Microsoft) and Statview (Abacus, Berkeley, CA). Relative protein signal was measured as the sum of pixel values in a defined ROI, corrected for background, exposure time, and radioactive decay. Values reported are averages of 3–7 SuC for each time point for each ROI.

**Protein Turnover in Electrically Inactive Presynapses**—Adult Syrian hamsters were intracocularly injected with 2 µl of [35S]Met/Cys (as above) containing 300 µCi tetrodotoxin (Alexis Corp., La¨ufelfingen, Switzerland) to abolish RGC action potentials for 24 h (23, 24). Such activity blockade would substantially eliminate visually evoked (but not spontaneous) synaptic release from RGC termini. Control hamsters received a normal [35S]Met/Cys injection. Metabolically labeled, rapidly transported proteins were analyzed from hamsters (n = 5 each condition) sacrificed at 8 h post-injection as described above.

**Analysis of Protein Glycosylation**—Radiolabeled APP and APLP2 were immunoprecipitated and subjected to enzymatic digestion. LGN and SuC from hamsters sacrificed 4 h postinjection were pooled and homogenized in buffer (50 mM Tris (pH 7.4), 155 mM NaCl, 5 mM EDTA, 1 mM Pefablock, 10 µg/ml aprotinin, 50 µg/ml leupeptin, 50 µg/ml pepstatin A). After homogenization, Triton X-100 (12.5 µM) and Nonidet P-40 (0.2%) was added to the homogenate mixture. The mixture was preclarified with hydrated protein A-Sepharose beads (Sigma).

**RESULTS**

**Radiolabeling of Presynaptically Targeted Proteins**—The central nervous system projection neuron of the retina, the RGC, sends an axon from the retina to synaptic target fields in the SC and LGN (Fig. 1B) (24). Since about 95% of RGC axons decussate in the adult Syrian hamster (25), our studies here focus only on proteins sent to target areas contralateral to the injected eye. Neosynthesis of protein in the eye and its subsequent axonal transport is the only significant source of radio-labeled protein in the target areas relevant to this study (Ref. 26; see footnote 2).

![Diagram](http://www.jbc.org)
Identification of Axonally Transported APSF Proteins—To differentiate highly homologous members of the APSF (Fig. 1A), a battery of antisera with well documented specificities (see “Experimental Procedures” and Table I) was used to probe the two-dimensional PVDF transfers. The positions and shapes of ROIs on autoradiograms matched corresponding spots on immunoblots with high precision. Six ROIs (Fig. 2A) were identified as immunopositive for one or more of these APSF-specific antisera. Fig. 2 summarizes multiple immunoblots. Antisera specific for APP and APLP2 immunoprecipitate radiolabeled proteins of appropriate molecular weight (see Fig. 4) and isoelectric point (not shown). This provides further evidence that overlap of spots on the immunoblots with matching ROIs on autoradiograms does indeed identify those ROIs as transported proteins.

**APP Proteins**—Polyclonal antibody (pAb) CT15 (APP C terminus) immunoreactivity on immunoblot ECL films precisely overlapped three ROIs (Fig. 2B, b–d) on autoradiogram film from hamsters sacrificed at 4 h (Fig. 2B). These ROIs on 4-h storage phosphor images matched congruent (but less intense) ROIs on 8 and 12 h storage phosphor images when viewed with contrast enhancement in pseudocolor mode (see Fig. 3). The three CT15-positive ROIs (b–d) were also detected by pAb GID (APP N terminus; Fig. 2C) but were not detected by other antisera specific for the other members of the APSF, including CT12 (APLP2 C terminus, Fig. 2D), CT11 (APLP1 C terminus, Fig. 2E), or D2-I (APLP2 N terminus, not shown). These three ROIs were also immunonegative for pAb R7 (KPI-APP, not shown). These new data, when taken together with other previously published findings (see “Discussion”), lead to the conclusion that ROIs c–e represent full-length variants of APP that are fast axonally transported to presynaptic terminals. ROI f (Fig. 2A) was immunopositive for the APP N-terminal-specific antiserum, GID, which also recognized ROIs b–d (Fig. 2C). However, ROI f was immunonegative for all APSF C-terminal antibodies (CT11, CT12, and CT15; Fig. 2, B–D) and for D2-II (not shown). The most parsimonious explanation for these new data and other previously published evidence (see “Discussion”) is that ROI f represents an APP variant lacking the C terminus.

**APLP2 Proteins**—Immunoreactivity for pAb CT12 (APLP2 C terminus) precisely overlapped with ROIs a and e (Fig. 2D). These two ROIs were also detected by pAb D2-II (not shown). ROIs a and e were not detected by any of the other antisera specific for the other members of the APSF, namely CT15 (Fig. 2F), GID (Fig. 2C), and CT11 (Fig. 2E). However, ROIs a and e differed in immunoreactivity in that ROI a was immunopositive for the KPI domain-specific antiserum R7 (not shown), whereas ROI e was immunonegative. These data suggest that ROIs a and e represent full-length variants of APLP2, resulting from expression of two distinct APLP2 isoforms, one with the KPI-domain (a) and one without the KPI domain (e).

**APLP1 Proteins**—pAb CT11 showed no reactivity in the adult tissues used in these studies for any radiolabeled ROIs, indicating that axonal transport of APLP1 to mature presynaptic termini is nonexistent or undetectable by our method (Fig. 2E).

**Quantitation of APP/APLP2 Levels and Turnover**—A visual qualitative impression of the protein turnover is given in a series of storage phosphor images in Fig. 3A. Levels of radiolabeled APP and APLP2 species transported to presynaptic termini of a single SuC, as represented by ROIs a–f, were quantified from storage phosphor images as the sum of pixel values in the individual ROIs. These sums from individual SuCs were grouped and averaged for post-injection time points. The averages were in turn scaled to maximum raw values for each ROI at each time point and graphed (Figs. 3, B and C). Three APP species (ROIs b–d; Fig. 3B) and two APLP2 species (ROIs a and e; Fig. 3C) are prominent among the newly synthesized proteins, arriving at the presynaptic terminus in the earliest wave of fast axonal transport. Visual examination of time course graphs shows that the levels for ROIs a–e peak at 4 h, after which time they decay with similar lifetimes. Table II documents that for the five fastest APSF ROIs, APP (ROIs b–d) and APLP2 (ROIs a and e) contribute equally to the total APSF protein species, which reach the presynaptic terminus by fast axonal transport. Of these five ROIs, a single APLP2 species (ROI a) contributes more than double any other species.

To more precisely describe protein lifetime in the presynaptic terminus, we quantified turnover rate for each ROI using three mathematically independent methods. First, decay from the peak value was fit by a single exponential curve, giving time constants of decay (decay to 37% of peak) ranging from 2.1 to 9.6 h (Table II). Second, simple linear decay from peak value to 50% peak level was calculated. These 50% linear decays ranged from 3.1 to 6.8 h (Table II). Finally, the transport curves were integrated, and the time by which 50% of total transported material had accrued was calculated. Subtracting 3.5 h (to estimate protein arrival time) from this time gives the time taken for eliminating 50% of transported material (presynaptic half-life). These half-lives ranged from 2.6 to 4.0 h (Table II). All three parameters are consistent in their ranking of turnover rate. Visual examination of time course graphs shows that the levels for ROIs a–e peak at 4 h, after which time they decay with similar lifetimes. Table II documents that for the five fastest APSF ROIs, APP (ROIs b–d) and APLP2 (ROIs a and e) contribute equally to the total APSF protein species, which reach the presynaptic terminus by fast axonal transport. Of these five ROIs, a single APLP2 species (ROI a) contributes more than double any other species.
aptic termini (Fig. 3B; Table II). It is also interesting that the peak of ROI $f$ (Table II) is significantly larger than the peaks of the other APP-ROIs (b–d). In fact, the sum of the peaks of ROIs b–d (at 4 h, Table II) accounts for 47% of the peak of ROI $f$ (at 8 h), whereas the sum of the integrated areas of time course curves (Fig. 3B) for ROIs b–d (Fig. 3B) accounts for 43% of the integrated area of ROI $f$. Thus, approximately one-half of ROI $f$ may in fact be more slowly transported than ROIs b–d (see “Discussion”). On the other hand, the large standard error of the peak value of ROI $f$ may, unfortunately, distort a closer quantitative match between ROIs b–d and ROI $f$. Nonetheless, these new data strongly suggest that a significant fraction of ROI $f$ could be due to presynaptic proteolytic processing of other APP species (ROIs b, c, and/or d). Finally, no putative cleavage fragments for APLP2 ROIs a or c were observed. This is noteworthy in that ROI a is highly abundant, and thus we would have expected to easily detect C-terminal fragments with pAb CT12 and N-terminal fragments with pAb D2-II.
Digestion of APLP2 with chondroitinase AC or chondroitinase ABC failed to show any bandshift (Fig. 4E; see Ref. 9), although the enzyme-treated material showed an intensification in two separate trials with both enzymes. The same pattern was seen for APP treated with the same chondroitinases (not shown). The band intensification after enzyme treatment likely results from digestion of CSGAG and dermatan sulfate glycosaminoglycan from large, unresolved APSF species that are axonally transported to RGC presynaptic termini. Thus, these data show that radiolabeled APP and APLP2 species (ROIs f–g) arriving at RGC presynaptic terminals are sialylated and N- and O-glycosylated, and that some also carry CSGAG and/or dermatan sulfate glycosaminoglycan.

**Discussion**

We examined APSF proteins that were conveyed by fast axonal transport to RGC presynaptic termini in the Suc and LGN of the Syrian hamster. Our results show that as early as 4 h after metabolic radiolabeling, several newly synthesized, electrophoretically distinct forms of APP and APLP2 reach peak levels in RGC-terminal regions consistent with transport in the most rapid phase of axonal transport. These APP and APLP2 proteins are quantitatively reduced by 50% 3–5 h after reaching RGC termini and after another 4 h were reduced to 10–25% of the peak levels at 4 h. These residence times for intact APP and APLP2 proteins in presynaptic termini are among the shortest seen for fast-transported presynaptic proteins, including cell adhesion molecule L1, the neural cell adhesion molecule (NCAM), cysteine string protein, SNAP25, and other uncharacterized proteins, some of which persist intact for days. A single exception to this pattern (ROI f, see “Results”) was detected, an APP species that peaks 8 h post-labeling and decays to 50% peak value by 12 h post-labeling. Finally, we note that a third member of the APSF, APLP1, was not detected among the radiolabeled proteins that are fast axonally transported to RGC presynaptic terminals.

In this system, maintaining constant levels of APP and APLP2 requires that the kinetics of delivery of APP and APLP2 to presynaptic termini be matched to the kinetics of their rapid removal. Rapid turnover kinetics for nonhuman APP has been documented for neuronal and nonneuronal cells in vitro (27). In these systems, rapid turnover of APP is due largely to a proteolytic a-secretase activity at the cell surface (28). Human and nonhuman APP can also be subject to two other proteolytic activities, b- and y-secretases, which together act to liberate the amyloidogenic peptide bA4 (1, 2). It is not known whether analogous a-, b-, or y-secretase activities exist in hamster. However, the present results, when taken with other previously published data, are consistent with a constitutive proteolysis of APP at the presynaptic terminal surface. First, one APP-species, ROI f, differs in its transport and turnover kinetics from all other APP proteins in that its peak accumulation is delayed and occurs during the period of reduction of the other APP proteins. Second, the amount of ROI f detected quantitatively is sufficient to account for all other presynaptic APPs that we measured. Third, although the other APPs are enriched in the membranous fraction (16), ROI f corresponds to an APP enriched in the soluble fraction (16) that is tyrosine-sulfated (29), a modification common to secreted proteins (30). Fourth, ROI f is not immunoreactive with C-terminal APP antibodies (present results) but is detected by N-terminal APP antibodies (Ref. 16 and present results). Finally, ROI f is the smallest of all APP proteins but still large enough to represent the secreted N-terminal portion of APP (28). Thus, all biochem-

---

**Fig. 4. Enzymatic digestion of carbohydrate moieties on immunoprecipitated APP (A and B) and APLP2 (C–F) from presynaptic terminals.** A and C, 1st lane, control; 2nd lane, N-glycanase (N-glyc) digestion. B and D, 1st lane, control; 2nd lane, neuraminidase (Neuram) digestion; 3rd lane, O-glycanase (O-glyc) digestion after neuraminidase digestion. E, 1st lane, control; 2nd lane, digested with chondroitinase (Ch AC). F, 1st lane, control; 2nd lane, digested with chondroitinase ABC.

---

**Effects of Synaptic Activity on APP/APLP2 Turnover**—Whether turnover kinetics for synthetically targeted APP/APLP2 species were dependent on neurotransmitter release mechanisms was tested by blocking action potential generation in RGC axons and measuring ROIs f–g 8 h post-radiolabeling. Our reasoning was that were turnover of APP and APLP2 activity-dependent, then ROIs f–g would remain elevated at 8 h rather than falling from peak levels at 4 h. Furthermore, we reasoned that were any ROI f derived from activity-dependent turnover of APP, that the peak of ROI f at 8 h would be diminished by activity blockade. Quantitative analysis of these data showed, however, that relative to control injection, tetrodotoxin injection did not raise ROIs f–g nor lower ROI f 8 h after radiolabeling (p value range 0.19–0.41, one-tailed t tests), i.e. all APP and APLP2 species appear to show the same transport and turnover kinetics in tetrodotoxin-injected animals as in control animals. Global level of protein transported during action potential blockade was not different from control levels (p > 0.23, t test), ruling out the possibility that tetrodotoxin injection has a global effect on transport kinetics per se. These data strongly suggest that axonal transport and presynaptic turnover kinetics of APP and APLP2 are independent of action potential conduction and mechanisms of visually evoked release of neurotransmitter.

**Glycosylation of Synaptically Targeted APP and APLP2**—Radiolabeled proteins were purified by immunoprecipitation, reacted with glycosidases, separated by one-dimensional polyacrylamide gel electrophoresis, and transferred to PVDF. Examination of autoradiograms for bandshift shows that APP undergoes a 1.0-kDa downward shift after treatment with N-glycanase (Fig. 4A). APP undergoes a 4.2-kDa downshift after neuraminidase treatment (Fig. 4B), and further treatment of the same sample with O-glycanase (Fig. 4C). APP undergoes a downshift of 2.2 kDa (Fig. 4D); subsequent O-glycanase treatment produces a further downshift of 0.8 kDa (Fig. 4D).

ical data concerning ROI $f$ are consistent with it being a soluble, N-terminal cleavage fragment of APP, for which the most prominently reported origin is liberation from the membrane by $\alpha$-secretase and/or $\beta$-secretase activity. Thus, we propose that one locus for such proteolysis is the presynaptic terminal membrane.

Both $\alpha$- and $\beta$-cleavage of APP has been reported in the optic nerve of rabbit (15, 31), although those data suggested that the proteolysis of neuronal APP occurred at the axolemma. Were proteolysis to occur in the transport vesicle in the axon, the C-terminal lacking fragment of APP could continue to undergo fast axonal transport to the presynaptic terminus and thus contribute to signal in ROI $f$ in excess of the local proteolysis, which peaks at 8 h post-radiolabeling. The fate of this fragment, whether locally produced or transported, is rapid elimination either by continued proteolysis, exocytosis, or retrograde transport or some combination of these events.

We have not been able to further trace the proteolytic degradation of APP or any APLP2 species because no other protein fragments with sizes and immunoreactivities consistent with APP or APLP2 proteolysis have been detected in our system. If such proteolysis is occurring, it may be either too rapid to detect with our protocol or accompanied by rapid endocytosis and retrograde transport away from the terminal arbor regions (32). A putative consensus clathrin binding site in C-terminal sequences of APP and APLP2 led us to speculate that APP and APLP2 turnover could be driven by coupling between activity-dependent exocytosis of neurotransmitter and clathrin-mediated endocytosis. We found, however, that APP and APLP2 turnover kinetics were normal even in the complete absence of evoked transmitter release from RGC axon terminals. An alternative interpretation is that residual spontaneous transmitter release in activity-blocked termini is sufficient to drive APP and APLP2 turnover. Nevertheless, this finding indicates that turnover mechanisms of APP and APLP2 are independent of actional potential conduction in the presynapse, even though APP may cycle with vesicular traffic at the presynapse (17, 33). However, it has been reported recently that APP colocalizes with a novel vesicle class in synaptosomal preparations that is distinct from the membrane associated with neurotransmitter vesicles (34). Thus, APP and APLP2 may reside in a membrane pool in the presynapse, which cycles independently of the pool involved in transmitter release.

Were human APP and APLP2 in nerve terminals subject to the same synthesis, transport, and turnover kinetics as we document for hamster, we predict that any perturbation of axonal transport or protein processing would rapidly lead to abnormal deficits and/or accumulations of APP and/or APLP2 in various cellular compartments. For instance, diminution of APP proteolysis would be predicted to lead to robust accumulation of intact APP at the presynaptic terminus. Alternatively, reduction of axonal transport to nerve terminals would lead to rapid depletion of APP at the nerve terminals but abnormally high accumulation in other cellular compartments where $\beta$A4 production may be favored (35). Although this latter scenario could have the potential for fueling altered $\beta$A4 production, as is observed for Alzheimer’s disease, it is also consistent with depletion of APP at the presynaptic terminal. Given our incomplete understanding of APP function in the neuron, it is difficult to predict the consequences to synaptic function during acute or chronic depletion of APP in presynaptic terminals. However, available evidence suggests that APP participates in synaptic function. For example, constitutive production of mutant APP (in the absence in normal APP) in transgenic animals has negative consequences for learning (36), and this is consistent with synaptic dysfunction due to aberrant APP function. In addition, other evidence suggests that APP turnover may have important consequences for various synaptic plasticities. For instance, it has been reported that secreted APP directly activates neuronal K$^+$ conductances on hippocampal neurons in culture (37), and that its application to hippocampal slice preparations modulates the expression of activity-induced depression and potentiation (38).

Expression patterns of APLP2 proteins show developmental regulation and linkage to axonal outgrowth and synapse formation in the retinal projection (16) and in other systems (39–41). The present in vivo data may contribute to understanding the function of neuronal APP and APLP2 in developing and mature neuronal circuits. For example, we find that presynaptically targeted APP and APLP2 proteins in an adult central nervous system pathway have carbohydrate modifications predicted on the basis of putative consensus glycosylation sequences. The present data show that, in addition to NCAM, APP and APLP2 also carry sialic acid to the presynaptic terminal membrane. At the synapse, NCAM and L1 have putative adhesive and signal transduction properties (42) that may regulate some synaptic plasticities and that may be modified by carbohydrate side-chain interactions (43, 44). It is interesting to speculate whether glycosylation of APLP2 proteins results in analogous modulation of adhesive and signaling properties. In fact, APLP2 proteins can potentially mediate transmembrane signaling via interactions with G-proteins (45) or the cytoskeleton (46, 47). A role for CSGAG modifications in structural plasticity in the adult central nervous system is suggested by the finding that nascent axons of the primary olfactory projection of adult rat express APLP2, which carries CSGAG (41). Finally, use of APP- and APLP2-specific antisera now shows that axonally transported APLP2 (and not APP) contains a KPI domain (11), and this corrects a previous conclusion (29) due to cross-reactivity of monoclonal antibody 22C11 for APP and APLP2 (5). The presence of a serine protease inhibitor domain such as the KPI on APLP2 at mature synapses reinforces data suggesting that extracellular serine proteases are involved in some forms of activity-dependent plasticity (48). However, evidence that a KPI-homologous peptide can block neuronal calcium channels (49) suggests another mechanism by which the KPI domain may modulate synaptic function. The possibility that APP and APLP2 may mediate some aspects of mature synaptic function underscores a prevalent theme in cellular neurobiology, namely that common sets of proteins are exploited by those cellular processes that control development of neuronal connections and those that maintain mature circuits.

Acknowledgments—We thank L. DiGiambardino for constant support, K. Rodolfo and R. Hassig for assistance in some of the metabolic labeling experiments, J. Shioi for generous contribution of the R7 antibody, and G. Cole for generous contribution of the GID antibody.

REFERENCES

Presynaptic Transport and Turnover Kinetics for APP and APLP2

Post-translational Processing and Turnover Kinetics of Presynaptically Targeted Amyloid Precursor Superfamily Proteins in the Central Nervous System
Alvin W. Lyckman, Anna Maria Confaloni, Gopal Thinakaran, Sangram S. Sisodia and Kenneth L. Moya

doi: 10.1074/jbc.273.18.11100

Access the most updated version of this article at http://www.jbc.org/content/273/18/11100

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 51 references, 24 of which can be accessed free at http://www.jbc.org/content/273/18/11100.full.html#ref-list-1