Differential Effects of the Swedish Mutant Amyloid Precursor Protein on β-Amyloid Accumulation and Secretion in Neurons and Nonneuronal Cells*

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Expression of the Swedish ΔNL mutation in the β-amyloid precursor protein (APPΔNL) dramatically increases Aβ generation in nonneuronal cell lines, although it is unclear whether intracellular levels of β-amyloid (Aβ) are also elevated after APPΔNL expression. Furthermore, the effects of expressing APPΔNL in neurons on the production and secretion of Aβ(1–40) and Aβ(1–42) are unknown. To address these issues, we examined the generation of both intracellular and secreted Aβ(1–40) and Aβ(1–42) in human neuronal NT2N cells, in primary rat astrocytes, and in Chinese hamster ovary cells engineered to express wild-type APP or APPΔNL using a recombinant Semliki Forest virus expression system. Expression of APPΔNL led to a marked increase in APPβ and the C-terminal fragment containing the entire Aβ sequence (C99) in all cells tested. However, a dramatic elevation of intracellular and secreted Aβ(1–40) and Aβ(1–42) was seen only in astrocytes and Chinese hamster ovary cells. The ΔNL mutation did not cause a significant increase in intracellular or secreted Aβ(1–40) or Aβ(1–42) in NT2N cells. Since NT2N cells expressing APPΔNL accumulate much higher levels of C99 than cells expressing wild-type APP, we conclude that the rate-limiting step in Aβ production could be the further processing of C99 by γ-secretase in these cells. These results show that the Swedish ΔNL mutation causes nonneuronal cells to process APP via pathways more in common with the metabolism of wild-type APP in neurons.

The 4-kDa amyloid β peptide (Aβ) is the principal proteinaceous component of senile plaques, the hallmark pathological feature of Alzheimer’s disease (AD). The Aβ peptide varies in length from 39 to 43 amino acids (1–5) and is derived from post-translational cleavage of the amyloid precursor protein (APP) (6–9). A variety of proteolytic pathways have been described for the processing of APP, but not all of them result in the production of full-length Aβ. The utilization of this pathway appears to be preferred by transfected nonneuronal cells expressing wild-type APP (APPwt), since the N-terminal ectodomain containing the first 17 amino acids of Aβ (i.e. APPα) and p3, the Aβ fragment beginning at amino acid residue 17 of Aβ, are recovered at high levels from media conditioned by these transfected nonneuronal cells (13, 14).

Processing pathways that result in the constitutive production of Aβ have also been identified, although their utilization is relatively minor in nonneuronal cells (13, 15, 16). Cleavage of APP by β-secretase at the N terminus of the Aβ sequence releases a soluble N-terminal fragment (APPβ) and generates a C-terminal fragment (C99) that contains the entire Aβ sequence. C99, but not APPβ, has been recovered from transfected nonneuronal cells expressing high levels of APPwt (15, 17). A second proteolytic activity termed γ-secretase, cleaves APP at the C-terminal end of the Aβ sequence, releasing Aβ(1–40) or Aβ(1–42) (18). Although secreted Aβ(1–40) and Aβ(1–42) are present in media conditioned by APPwt-transfected nonneuronal cells, intracellular Aβ has not been detected (19–21).

Unlike transfected nonneuronal cells expressing APPwt, postmitotic neurons such as human NT2N cells predominantly utilize the β-secretory pathway at the expense of the α-secretory pathway to process endogenous APP (22–24). For example, NT2N cells secrete much higher levels of Aβ(1–40) and Aβ(1–42) than p3 (23, 24). In addition, intracellular Aβ(1–40) and Aβ(1–42), but not p3, can be recovered in NT2N cells before their detection in the culture medium, suggesting an intracellular location for β-secretase. Indeed, intracellular APPβ has recently been identified in NT2N cell lysates. By contrast, intracellular APPα has not been detected in NT2N cells (23, 24). At least one intracellular location for β-cleavage is within the endoplasmic reticulum/intermediate compartment (ER/IC) (24, 25). Importantly, APP processed by the

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1 The abbreviations used are: Aβ, β-amyloid; AD, Alzheimer’s disease; Aβ(1–40), Aβ containing 40 amino acid residues; Aβ(1–42), Aβ containing 42 amino acid residues; APP, β-amyloid precursor protein; APPwt, wild-type human APPwt protein; APPΔNL, human APPwt protein bearing the Swedish double mutation; APPs, N-terminal ectodomain of APP derivatives; APPα, α-secretase cleaved N-terminal ectodomain of APP; APPβ, β-secretase-cleaved N-terminal ectodomain of APP; APPΔNL, β-secretase-cleaved N-terminal ectodomain of APPΔNL; p3, Aβ fragments cleaved at amino acid residues 16 and 17 of Aβ; C99, C-terminal fragment containing the entire Aβ sequence; C83, C-terminal fragment containing only p3; NT2N cells, neurons derived from a human embryonal carcinoma cell line (NT2); CHO cells, Chinese hamster ovary cells; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethylglycine; ELISA, enzyme-linked immunosorbent assay; ER, endoplasmic reticulum; IC, intermediate compartment; SFV, Semliki Forest virus; PBS, phosphate-buffered saline; FBS, fetal bovine serum; MEM, modified Eagle’s medium.

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ER/IC pathway in NT2N cells produced only Aβ-(1–42) but not Aβ-(1–40) (25, 26). Thus, it is evident that APP processing is cell-type-specific and that neuronal cells process APP differently from nonneuronal cells.

Studies of a Swedish family with familial AD identified a double mutation immediately flanking the N terminus of the Aβ domain (APPANL (27)) that results in elevated plasma levels of Aβ-(1–40) and Aβ-(1–42) (28). Moreover, nonneuronal cells transfected with APPANL secrete three to six times more Aβ-(1–40) and Aβ-(1–42) than cells transfected with APPwt (29, 30). Unlike nonneuronal cells expressing APPwt, nonneuronal cells transfected with APPANL produce intracellular Aβ and APPβANL (19–21, 31, 32), suggesting that the ΔNL mutation diverts APP into a processing pathway that resembles that found in mitotic neurons. To test this hypothesis, we compared the processing of APPwt and APPANL in postmitotic NT2N cells, in primary rat astrocytes, and in Chinese hamster ovary (CHO) cells. We found that APP is processed in a distinct manner in NT2N neurons and that the ΔNL mutation does not cause an increase in secreted or intracellular Aβ despite causing an increase in APPANL and C99 in these cells. However, this mutation does lead to greatly increased levels of intracellular and secreted Aβ-(1–40) and Aβ-(1–42) in both primary astrocytes and nonneuronal cell lines. These data provide evidence that the consequences of this familial AD-associated mutation on APP processing in nonneuronal cells is to increase the utilization of the β-secretary pathways at the expense of the α-secretory pathway such that they resemble the processing pathways in mitotic neurons. Finally, we also provide evidence that γ-secretase, but not β-secretase, is a rate-limiting step in the production of Aβ in NT2N neuronal cells.

EXPERIMENTAL PROCEDURES

Antibodies—The epitopes recognized by the various APP-specific antibodies used in this study are depicted in Fig. 1. The goat polyclonal anti sera to the ischemic core of the brain were generously provided by Dr. T. Golde (University of Pennsylvania, Philadelphia, PA) (34), and monoclonal antibodies BAN-50, BC-05 and BA-27 were the generous gift of Dr. N. Suzuki of Takeda Pharmaceuticals (San Francisco, CA) (16, 31). The rabbit polyclonal antibodies used in this study are depicted in Fig. 1. The goat polyclonal antibodies provided by Dr. T. Golde (University of Pennsylvania, Philadelphia, PA) (34), and monoclonal antibodies BAN-50, BC-05 and BA-27 were the generous gift of Dr. N. Suzuki of Takeda Pharmaceuticals (San Francisco, CA) (16, 31). The rabbit polyclonal antibodies used in this study are depicted in Fig. 1. The goat polyclonal antibodies used in this study are depicted in Fig. 1. The goat polyclonal antibodies used in this study are depicted in Fig. 1. The goat polyclonal antibodies used in this study are depicted in Fig. 1. The goat polyclonal antibodies used in this study are depicted in Fig. 1. The goat polyclonal antibodies used in this study are depicted in Fig. 1. The goat polyclonal antibodies used in this study are depicted in Fig. 1. The goat polyclonal antibodies used in this study are depicted in Fig. 1.

Plasmid Constructs and Virus Production—The Semliki Forest virus (SFV) expression system was used to express APPwt and APPwt bearing the Swedish mutation (APPANL) in a variety of cell types. The SFV virus was transfected into SFV-1, pSFV-Helper 2, and pSFV3-lacZ was purchased from Life Technologies, Inc. (37). The pSFV-1 polylinker was modified to include Clal as an unique cloning site. CDNA clones encoding APPwt and APPANL were provided by Dr. T. Golde (University of Pennsylvania, Philadelphia, PA) (29). APPwt was ligated into the modified pSFV1 at the restriction enzyme sites BamHI and Clal. APPANL was blunt-end ligated into pSFV1 at the restriction enzyme site SmaI. Recombinant virus vectors were produced as described previously (37). Briefly, recombinant and helper plasmids were linearized with Spe I and used as a template for the production of RNA using the T7 polymerase. Approximately 25 μg of recombinant and helper RNA were then electroporated into 1 × 10^8 BHK-21 cells in PBS. The electroporated cells were incubated for 24 h in 24 ml of complete Glasgow minimal essential medium (Life Technologies, Inc.) and subsequently, the viral supernatant was harvested and stored frozen at −70 °C.

Virus titer was determined by infection of BHK-21 cells with 10-fold serial dilutions of recombinant virus. After 16 h of infection, cells were fixed with ice-cold methanol, 5% acetic acid and blocked with 5% FBS in Dulbecco’s PBS. The cells were subsequently incubated with antibody Karen followed by horseradish peroxidase-conjugated rabbit-antigoat immunoglobulin. Staining was developed with 0.5 mg/ml 3,3′-diaminobenzidine in 0.1 M Tris, pH 7, 0.01% Triton X-100, 10 mM imidazole, and 0.01% H_2O_2.

Cell Culture—CHO Fros were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and cultured in a minimal essential medium (α-MEM; Life Technologies, Inc.) supplemented with 10% FBS (HyClone Laboratories, Inc., Logan, UT), 2 mM glutamine, 100 units/ml penicillin G sodium and 100 μg/ml streptomycin sulfate. BHK-21 cells were obtained from the ATCC and cultured in Glasgow minimal essential medium (Life Technologies, Inc.) supplemented with 10% FBS. The mouse primary astrocytes were purchased from the Life Technologies, Inc. (35, 36).

RESULTS

Expression of Human APPwt and APPANL in Neuronal and Nonneuronal Cells—To express wt and mutant forms of APP, we utilized recombinant SFV vectors. SFV efficiently expresses...
proteins in NT2N cells without cytopathic effects for at least 48 h (data not shown), and APP expressed by recombinant SFV vectors is expressed normally in a variety of cell types including CHO cells and primary rat astrocytes (24, 25, 42). Furthermore, expression of APP in rat hippocampal neurons by SFV vectors results in amyloidogenic processing of APP (43, 44). Thus, SFV vectors provide an opportunity to express APP under conditions that result in normal proteolytic processing.

To determine if there are cell-type-dependent differences in the processing of wt and mutant APP, we expressed APPwt and APP\textsuperscript{DNL} in CHO cells, NT2N neurons, and primary rat astrocytes. A recombinant SFV vector that expresses lacZ served as a control. APP expression was optimized for each cell line by varying the length of infection. After 24 h infection (16 h for the CHO cells), cell lysates and media were collected and analyzed by SDS-PAGE for the presence of APP and secreted APP\textsubscript{S} by immunoblotting with antisera directed against the APP ectodomain (Figs. 1 and 2). Comparable amounts of full-length APP were detected in the cell lysates with both endoglycosidase H-sensitive (\textit{lower band}) and resistant (\textit{upper band}) forms being present in CHO cells and astrocytes. Cells expressing APP\textsuperscript{ANL} expressed slightly reduced amounts of endoglycosidase H-resistant APP (data not shown). By contrast, the APP recovered from NT2N cell lysates was predominantly endoglycosidase H sensitive (see also Ref. 24). The amount of APP\textsubscript{S} secreted into the media was largely independent of the cell type and whether APPwt or APP\textsuperscript{ANL} were expressed. This experiment demonstrates that APP was readily expressed and detected in all three cell types using the SFV system and that both APPwt and APP\textsuperscript{ANL} were processed, resulting in the secretion of APP\textsubscript{S}.

Effects of the APP\textsuperscript{ANL} Mutation on A\textsubscript{\textbeta}-(1–40) and A\textsubscript{\textbeta}-(1–42) Secretion—To assess the effect of the APP\textsuperscript{ANL} mutant on A\textsubscript{\textbeta} secretion in the different cell types, we infected human NT2N neurons, undifferentiated NT2- cells (from which NT2N neurons are derived), CHO cells, and primary rat astrocytes with SFV vectors expressing either APPwt, APP\textsuperscript{ANL}, or lacZ. At 16 (CHO cells) or 24 h (NT2N cells and astrocytes) post-infection, the media was harvested, and cells were lysed as described. Media and lystate, normalized for total APP expression, were resolved on a 7.5% polyacrylamide gel and immunoblotted with a polyclonal antibody to APP (Karen). Molecular mass standards are indicated in kDa.

Effects of the APP\textsuperscript{ANL} Mutation on A\textsubscript{\textbeta}-(1–40) and A\textsubscript{\textbeta}-(1–42) Secretion—To assess the effect of the APP\textsuperscript{ANL} mutant on A\textsubscript{\textbeta} secretion in the different cell types, we infected human NT2N neurons, undifferentiated NT2- cells (from which NT2N neurons are derived), CHO cells, and primary rat astrocytes with SFV vectors expressing either APPwt, APP\textsuperscript{ANL}, or lacZ. At 16 (CHO cells) or 24 h (NT2N cells and astrocytes) post-infection, the media was harvested, and cells were lysed as described. Media and lystate, normalized for total APP expression, were resolved on a 7.5% polyacrylamide gel and immunoblotted with a polyclonal antibody to APP (Karen). Molecular mass standards are indicated in kDa.

The total amount of APP\textsubscript{S} expressed in each cell line as determined by quantitative Western blotting. Despite the diversity of cell types used and the fact that the absolute amount of A\textsubscript{\textbeta} produced by each cell line varied widely, we found that expression of APP\textsuperscript{ANL} caused a 4–8-fold increase in the levels of secreted A\textsubscript{\textbeta}-(1–40) and A\textsubscript{\textbeta}-(1–42) compared with APPwt in primary rat astrocytes, CHO cells, and NT2- cells (Fig. 3). In all these cells, the ratio of A\textsubscript{\textbeta}-(1–40) to A\textsubscript{\textbeta}-(1–42) was approximately 10:1, consistent with other reports (23, 36). In marked contrast, expression of APP\textsuperscript{ANL} in NT2N neurons resulted in only a modest increase (0–35%) in both A\textsubscript{\textbeta}-(1–40) and A\textsubscript{\textbeta}-(1–42) secretion compared with APPwt (Fig. 3). By comparison to
the nonneuronal cells, the lack of enhancement in NT2N neurons was observed in seven of seven independent experiments. To determine if the modest increase in Aβ secretion from NT2N cells was merely a result of the overexpression APP, we measured the levels of secreted Aβ at 6 h post-infection when the expression of APP is 5–10-fold less than at 24 h post-infection. As expected, the levels of Aβ-(1–40) and Aβ-(1–42) secreted at 6 h was very low compared with that seen at 24 h. Nonetheless, expression of APPΔNl resulted in only a 28% increase in Aβ-(1–40) and a 30% decrease in Aβ-(1–42) at 6-h post-infection compared with APPwt (Fig. 4). Thus, even under conditions where APP processing levels were far from maximal, expression of APPΔNl in NT2N neurons did not cause the pronounced elevation of Aβ secretion observed in nonneuronal cells. Qualitatively comparable results were obtained in three independent experiments.

Effects of APPΔNl on Intracellular Aβ Production in CHO and NT2N Cells—To assess the effect of the APPΔNl mutation on intracellular Aβ-(1–40) and Aβ-(1–42) levels in neurons and a nonneuronal cell line, we expressed APPwt, APPΔNl, or lacZ in NT2N and CHO cells using SFV vectors. The cells were lysed 24-h post-infection and assayed for Aβ-(1–40) and Aβ-(1–42) by ELISA. In the NT2N cells, expression of APPΔNl resulted in only a 40% increase in intracellular Aβ-(1–40) and a 10% increase in Aβ-(1–42) compared with that seen with APPwt (Fig. 5). In contrast, expression of APPΔNl in CHO cells resulted in a 5-fold increase in intracellular Aβ-(1–40) and a 2-fold increase in Aβ-(1–42) APPwt (Fig. 5). Comparable results were obtained in four of four independent experiments. The small increase in intracellular Aβ observed in NT2N cells and the much larger increase seen in CHO cells correlated with the levels of Aβ secreted by these cell types (Fig. 3). Thus, the increased levels of Aβ observed in the media of CHO cells after expression of APPΔNl was a result of increased Aβ production rather than decreased turnover or loss following secretion.

Effects of APPΔNl on APPβ Production and Secretion—We next investigated the efficiency of β cleavage following APPΔNl expression in NT2N neurons, astrocytes, and CHO cells to determine if this step was rate-limiting for Aβ production in NT2N cells. Cells were infected with the appropriate SFV vector, cell lysates and media were collected, and aliquots of each were subjected to SDS-polyacrylamide gel electrophoresis and quantitative immunoblotting to measure total APP expression and APPβ secretion, respectively. To determine if the shift toward slightly lower molecular weight-secreted APPs seen in CHO cells, primary rat astrocytes, and NT2N cells after APPΔNl expression was due to increased levels of APPβ relative to APPα, we used antibodies specific for either APPα (6E10), APPβ (192), or APPβΔNl (192SW) (Fig. 1). We found that expression of APPΔNl led to a reduction in the amount of APPα secreted, although the level of reduction varied between the three different cell lines (Fig. 6). Concomitant with the reduction in secreted APPα, a large increase in the amount of secreted APPβ was observed from NT2N cells compared with the other cell types. By contrast, APPβ was below the level of detection in media collected from astrocytes and CHO cells expressing APPwt. The large increase in APPβ released from NT2N cells suggests that the rate-limiting step in Aβ production after APPΔNl expression is more likely to involve the γ-secretase cleavage.

![Figure 3](image-url)
Effects of APPΔNL Mutation on Intracellular APPβ and APPβΔNL Levels—To compare the effect of the APPΔNL mutation on the intracellular processing of APP in neuronal and nonneuronal cells, we examined the intracellular levels of APPβ and APPβΔNL in astrocytes, CHO, and NT2N cells after expression with either APPwt or APPΔNL. In all cells tested, intracellular APPβ was not detected after APPwt expression (data not shown), consistent with previous data showing that α-secretase cleavage of APP occurs at or near the plasma membrane (12, 15, 24). Furthermore, although APPβ was not detected in cell lysates of astrocytes and CHO cells expressing APPwt, intracellular APPβ was readily detected in NT2N neurons, consistent with their constitutive production and secretion of Aβ and APPβ (Fig. 7). However, expression of APPΔNL sharply increased the amount of detectable APPβΔNL in the NT2N cells, although it is evident that this antibody cross-reacts to a limited degree with full-length APPΔNL (see asterisk in Fig. 7). Significantly, intracellular APPβΔNL was detected in astrocytes and CHO cells after APPΔNL expression (Fig. 7). The increase in intracellular APPβΔNL in NT2N cells again suggested increased APP processing by the β-secretory pathway after expression of APPΔNL.

The Swedish Family Mutation Increases C99 Production Although Decreasing C83—The dramatic increase in APPβΔNL without an accompanying increase in Aβ levels in NT2N cells expressing APPΔNL suggested that either the processing by γ-secretase(s) is rate-limiting or that the carboxyl-terminal C99 fragment is rapidly degraded. To distinguish between these possibilities, we analyzed cell lysates prepared from CHO and NT2N cells expressing either APPwt or APPΔNL for C99 and C83 with the antibodies 369W and 6E10. Antibody 369W is specific for the carboxyl terminus of APP and thus recognizes both C99 and C83, whereas antibody 6E10 only recognizes C99 (Fig. 1). In CHO cells expressing APPwt, the C83 fragment was the predominant species detected (Fig. 8), whereas expression of APPΔNL lead to the production of the C99 fragment as well, consistent with increased β-secretase processing (Fig. 8). In contrast, the C99 fragment was detectable after APPwt expression in NT2N neurons (Fig. 8) at levels of approximately 30–50% of that of C83. When APPΔNL was expressed in the NT2N cells, there was a dramatic increase in the level of C99 with a concomitant decrease in C83 (Fig. 8). The amount of C99 pres-
The Swedish family mutation in APP increases intracellular APPβ. 1 × 10⁶ cells as indicated were infected with lacZ, wild-type, or mutant APP recombinant SFV. At 16 (CHO cells) or 24 h (NT2N cells and astrocyes) post-infection, the cells were washed and lysed. Cell lysate, normalized for total APP expression, was resolved on a 7.5% polyacrylamide gel and immunoblotted with antibodies as indicated. The asterisk designates full-length APP.

The increased processing by β-secretase(s) observed in all cell types after APPΔNL expression could be the result of several factors. At present, at least three different β-secretase processing pathways have been reported. The endosomal/lysosomal pathway, which processes APP after re-internalization from the cell surface into endosomes and lysosomes, is the most ubiquitous since both neurons and nonneuronal cells utilize this pathway to produce Aβ. However, the contribution of this pathway to the overall production of Aβ is relatively minor since nonneuronal cells transfected with APPwt produce mostly p3 and very little Aβ (15, 20, 47, 48). In addition, it is unlikely that APPΔNL expression increases Aβ production via this pathway, since the expression of an APPΔNL construct lacking the cytoplasmic tail, which eliminates re-internalization of cell surface APPΔNL, does not reduce Aβ secretion (31, 49). A second β-secretory pathway, active primarily in Golgi-derived vesicles, is more likely to process APPΔNL (31). Previous studies have suggested that nonneuronal cells utilize this pathway at the expense of the α-secretory pathway when APPΔNL is expressed (31). However, it is unclear whether Aβ(1–40) and Aβ(1–42) are both produced by this route, since a recently identified β-secretory pathway localized to the ER/IC results in exclusive production of Aβ(1–42) (25, 26). Since the secretion of both Aβ(1–40) and Aβ(1–42) is elevated in nonneuronal cells expressing APPΔNL, it is possible that both the Golgi-associated and the ER β-secretase pathways are affected by this mutation. Alternatively, it is possible that both Aβ(1–40) and Aβ(1–42) can be produced by Golgi-derived vesicles and that the ΔNL mutation only affects this pathway selectively.

The increased processing of APPΔNL by the β-secretase pathway in all cells tested coupled with markedly increased levels of Aβ production in all cells except NT2N neurons suggests that γ-secretase processing may be rate-limiting for Aβ production in NT2N cells by one of several mechanisms. For example, C99 may be more rapidly degraded in NT2N cells than in nonneuronal cells, precluding or minimizing the possibility of γ-secretase cleavage of C99 and concomitant Aβ production. However, it is unclear whether the observed increase in the C99 fragment after APPΔNL expression in NT2N cells. Another possibility is that the high levels of expression resulting from the SFV vector system saturates the γ-secretase. However, we found no difference in Aβ levels between NT2N cells expressing APPwt or APPΔNL at early time points when APP expression levels were low. In addition, De Strooper et al. (43) found that expression of APPΔNL in rat hippocampal neurons caused only a 2-fold increase in Aβ levels relative to APPwt as judged by immunoprecipitation. Although larger than the increase we observed in this study using a more quantitative ELISA approach, the increase in Aβ was still significantly less than the 5–10-fold increase observed in nonneuronal cells. Finally, the intracellular processing pathways utilized by the NT2N cells may be distinct from that of the nonneuronal cells (12, 13, 22, 23, 25). Thus, the possibility exists that the γ-secretase did not have access to the carboxy-terminal fragments generated when APPΔNL was expressed. Identification of the γ-secretase(s) will help distinguish these possibilities.
Several studies suggest that the cellular source of Aβ deposition in senile plaques in nonfamilial cases of AD is the neuron (50, 51). Our data supports this hypothesis because only neuronal cells constitutively generate Aβ. This production of Aβ is accomplished by the generation of intracellular APPβ and C99 in NT2N cells. By contrast, intracellular APPβ and C99 were absent from nonneuronal cells expressing APPwt. This suggests that commitment to neuronal differentiation results in the acquisition of the ability to utilize the β pathway of APP processing, a necessary prerequisite for Aβ production. However, expression of APPNL led to markedly increased production of Aβ, APPβ, and C99 in nonneuronal cells, indicating that the Swedish ΔNL mutation alters APP processing in nonneuronal cells such that APP is now processed in a more “neuronal” fashion. NT2N cells may not exhibit such marked alterations in APP and Aβ metabolism after APPNL expression, because they are already committed to processing APP in pathways that favor increased production of both intracellular and secreted Aβ. The relatively modest effects of this mutation on Aβ production in NT2N neurons raises the question as to what role neurons play in elevating Aβ levels in individuals with the ΔNL mutation. It is possible that in such individuals cell types other than neurons, such as astrocytes, may significantly contribute to the marked increases in central nervous system Aβ levels and the formation of senile plaques.

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