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β-Amyloid efflux mediated by p-glycoprotein

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Abstract

A large body of evidence suggests that an increase in the brain β-amyloid (Aβ) burden contributes to the etiology of Alzheimer's disease (AD). Much is now known about the intracellular processes regulating the production of Aβ, however, less is known regarding its secretion from cells. We now report that p-glycoprotein (p-gp), an ATP-binding cassette (ABC) transporter, is an Aβ efflux pump. Pharmacological blockade of p-gp rapidly decrease extracellular levels of Aβ secretion. *In vitro* binding studies showed that addition of synthetic human Aβ1–40 and Aβ1–42 peptides to hamster mdr1-enriched vesicles labeled with the fluorophore MIANS resulted in saturable quenching, suggesting that both peptides interact directly with the transporter. Finally, we were able to directly measure transport of Aβ peptides across the plasma membranes of p-gp enriched vesicles, and showed that this phenomenon was both ATP- and p-gp-dependent. Taken together, our study suggests a novel mechanism of Aβ detachment from cellular membranes, and represents an obvious route towards identification of such a mechanism in the brain.

Keywords: ABC transporter, Alzheimer's disease, β-amyloid, MDR1, membrane, p-glycoprotein.
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Alzheimer's disease (AD) is a neurodegenerative disorder whose pathological hallmarks include neurofibrillary tangles, senile plaques, and neuronal death. The neurofibrillary tangles contain paired helical filaments composed of hyperphosphorylated tau, while the senile plaques are comprised of an array of proteins deposited around a core of insoluble β-amyloid (Aβ) peptide (Cummings *et al*. 1998). The cause of neuronal death remains unknown but a considerable body of evidence suggests that it is secondary to an increase in the brain Aβ load (Selkoe 1999). Particularly compelling are data which derive from relatively rare cases of familial AD, in which mutations in any one of three genes [amyloid precursor protein (APP), presenilin 1, and presenilin 2] result in early onset AD accompanied by increased extracellular levels of the longer isoform of Aβ known as $A\beta_{1-42}$ (Hardy *et al*. 1999). Moreover, transgenic animals expressing these gene mutations recapitulate many of the features of AD, including amyloid plaques, cerebrovascular amyloid angiopathy, and neuronal cell death (Price *et al*. 1998). As a result, much effort has been devoted to understanding the molecular mechanisms involved in synthesis and degradation of Aβ.

It is well established that Aβ is constitutively produced by sequential endoproteolytic cleavage of APP by enzymes termed β- and γ-secretase (Selkoe 1999). The β-secretase cleavage site is located 28 amino acids away from the extracellular face of the membrane, while the γ-secretase cleavage site is located within the lipid bilayer. Moreover, γ-secretase cleavage occurs at multiple sites within the membrane spanning domain, with the dominant cleavage

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Abbreviations used: Aβ, β-amyloid; ABC, ATP-binding cassette; AD, Alzheimer's disease; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; p-gp, p-glycoprotein; PMA, phorbol ester; SDS, sodium dodecyl sulfate.

occuring 12 amino acids COOH-terminal to the extracellular face of the membrane resulting in production of the 40 amino acid Aβ peptide known as $A\beta_{1-40}$, and the less common cleavage occuring 14 amino acids COOH-terminal to the extracellular face producing the 42 amino acid version of Aβ known as $A\beta_{1-42}$. Thus, Aβ peptides are amphipathic, consisting of 28 hydrophilic amino acids and 12–14 hydrophobic amino acids.

The Aβ peptides are rapidly released from both neuronal and non-neuronal cells (Haas *et al*. 1992; Seubert *et al*. 1992; Shoji *et al*. 1992; Busciglio *et al*. 1993). However, the stretch of 12–14 hydrophobic amino acids at the COOH-terminus dictates that the peptide remains associated with the membrane following γ-secretase cleavage. In order to reconcile these discordant observations, we hypothesized that the final step in Aβ secretion requires active detachment of Aβ from the membrane. In considering mechanisms which might account for such a phenomenon, we were struck by the observation that selected members of the ATP-binding cassette (ABC) superfamily of transporters are responsible for the energy-dependent efflux of a variety of lipophilic and amphipathic molecules from cells (van Veen and Konings 1998; Kuchler and Thorner 1992; Croop 1998; Ambudkar *et al*. 1999; Yakushi *et al*. 2000). We hypothesized that an ABC transporter might be responsible for Aβ release from cells. We now provide evidence that the ABC transporter known as MDR1 is an Aβ efflux pump.

Materials and methods

Transient transfection of HEK293 cells

HEK293 cells stably transfected with APP harbouring the Swedish double mutation (K269sw, Citron *et al*. 1996) which results in an eight-fold increase in Aβ secretion as compared with wild-type cells, kindly provided by Dr Dennis Selkoe, Harvard Medical School), were grown on 100 mm plates to ~ 70% confluency in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and geneticin (40 μg/mL). Transient transfections using the calcium phosphate method were carried out as described (Mills *et al*. 1997), and resulted in 80–90% transfection efficiency using β-galactosidase staining. In brief, the cells were exposed to 10 μg of cDNA encoding either human MDR1 (pHaMDR1, Pastan *et al*. 1988) or β-galactosidase (β-gal) for 9 h. The cells were then manually lifted from the plate bottoms using trituration and replated at a density of 2×10^6 cells/plate onto poly-D-lysine coated 60 mm dishes and maintained for a total of 48 h post-transfection prior to measurement of Aβ in the medium.

Wild-type HEK293 cells (kindly provided by Dr Lynn Raymond, University of British Columbia) were cotransfected with 3 μg of pCDNA3.1APPsw (a gift from Active Pass Pharmaceuticals, Vancouver, Canada) and 3 μg of either pHaMDR1 or MRP1 (Grant *et al*. 1994; kindly provided by Drs Roger Deeley and Susan Cole, Queen's University), using Lipofectamine (Gibco Life Technologies, Burlington, Ontario, Canada). The cells (1.5×10^4) were plated onto 60 mm dishes for 18 h and transfected following the manufacturer's protocol (resulting in 90–100% transfection efficiency using β-gal staining), and extracellular Aβ was measured 48 h later.

Drug treatment and detection of extracellular β-amyloid

For all drug treatments, experiments were performed 48 h post-transfection. Cells were washed once with warm phosphate-buffered saline (Sigma-Aldrich, Oakville, Ontario, Canada) and 1 mL of fresh DMEM (Gibco) containing either vehicle [0.01% DMSO (v/v); Sigma] or the indicated concentrations of RU486 [Mifepristone, 17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-prop-1-ynyl estra-4, 9-diene-3-one; RBI, Natick, MA, USA] or RU49953 [17β-hydroxy-11β,17α-(4-dimethylaminophenyl)-17α-prop-1-ynyl estra-4,9-dien-3-one, kindly provided by Roussel-Uclaf, Romainville, France] was added for the indicated periods of time. For measurements of basal Aβ release, cells were washed as described above and replaced with 1 mL of DMEM for 1 h and both cells and media harvested. Cells were lyzed in an extraction buffer containing 1% Nonidet P-40, 1% sodium deoxycholate, 100 mM NaCl pH 7 supplemented with 1 μg/mL pepstatin A, 2 μg/mL leupeptin, and 2 μg/mL aprotinin. Non-detergent soluble lysate was spun down briefly at 5000 *g* at 4°C and 2 μL of the supernatant was removed from each sample for protein quantification using a bicinchonic acid assay (Pierce, Rockford, Il, USA). The media was subjected to trichloroacetic acid precipitation to isolate total secreted protein, the pellet was resuspended in Laemmli buffer, and normalized amounts of protein were resolved on 16.5% Tris-tricine sodium dodecyl sulfate (SDS) gels as described (Mills *et al*. 1997). Total Aβ was measured in western blots using either the N-terminal monoclonal antibodies WO-2 (Ida *et al*. 1996; a kind gift of Professor Konrad Beyreuther, Univ. Heidelberg, Germany) or 6E10 (Senetek Research, Maryland Heights, MO, USA). Using this method, we were able to measure 5 ng of synthetic Aβ (unpublished observations). Bands were visualized using ECL (Amersham, Piscataway, NJ, USA).

Detection of p-glycoprotein

To detect p-glycoprotein (p-gp), 100 μg of cellular protein was resolved on 7.5% polyacrylamide gels and transferred onto nitrocellulose. The monoclonal antibody C219 (ID Labs, Inc., London, Ontario, Canada), which recognizes an internal epitope of p-gp was used to probe the blot for p-gp. Bands were visualized using ECL (Amersham).

β-Amyloid peptide binding to purified hamster mdr1

Binding of Aβ peptides to mdr1 was studied using fluorescence quenching, as described previously for peptides and drug substrates (Liu and Sharom 1996; Sharom *et al*. 1998b, 1999). Briefly, highly purified mdr1 reconstituted into vesicles and labeled with MIANS was titrated with human synthetic $A\beta_{1-40}$ and $A\beta_{1-42}$ (RBI), and quenching of the fluorescence emission at 420 nm was monitored. The dissociation constant K_d was estimated by fitting the data to an equation describing interaction with a single class of binding site.

Modulation of mdr1 ATPase activity and drug transport by β-amyloid peptides

The ATPase activity of mdr1 in plasma membrane vesicles derived from the multidrug-resistant cell line CH^RB30 was measured as described previously (Sharom *et al*. 1995b) in the presence of increasing concentrations of $A\beta_{1-40}$ or $A\beta_{1-42}$. ATP-dependent

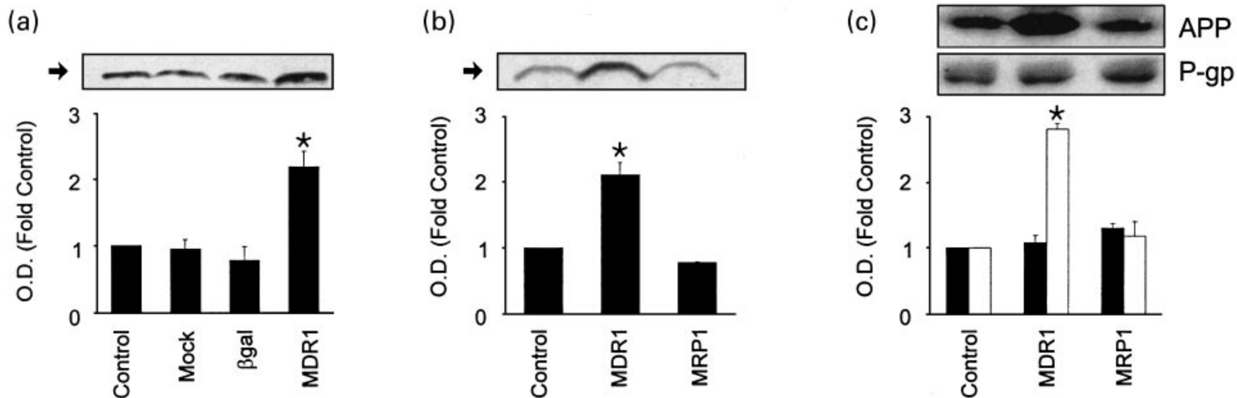

Fig. 1 Transient transfection of human *pHaMDR1* increases Aβ secretion. (a) K269sw cells were either not transfected (control), mock transfected using calcium phosphate precipitation without plasmid (Mock), or with either 10 μg β-galactosidase (βgal) or 10 μg human *MDR1* (MDR1). Forty-eight hours after transfection, the medium was changed and secreted Aβ was measured 1 h later. Transfection with *pHaMDR1* significantly increased basal Aβ secretion approximately twofold above control ($n = 3$, $*p < 0.01$). Western blotting was performed using the monoclonal antibody WO-2. The arrow indicates Aβ at approximately 4 kDa. (b) HEK293 cells were either not transfected (control), or transiently cotransfected using Lipofectamine with 3 μg APP695sw and either 3 μg human *pHaMDR1* (MDR) or 3 μg *pCDNA3.1MRP1* (MRP1) and Aβ measured in the extracellular medium. Western blot detection of Aβ was performed using the 6E10 monoclonal antibody. Again, MDR1 significantly increased Aβ secretion by ~three-fold over control while MRP1 did not show significant change ($n = 3$, $*p < 0.01$). (c) Total APP and p-gp from cellular extracts of transfections. APP was detected using 22C11 and p-gp was detected using C219. In cells transfected with *pHaMDR1*, total cellular APP levels were increased (white bars; $n = 3$, $*p < 0.01$), while no increases were observed in cells transfected with *pCDNA3.1MRP1*. Transfection with *pHaMDR1* did not significantly increase cellular p-gp expression (black bars, $n = 3$).

uptake of [^{3}H]-colchicine into CH^{R}B30 plasma membrane vesicles was determined by rapid filtration as outlined earlier (Sharom *et al.* 1995a, 1998b) in the presence of increasing concentrations of Aβ$_{1-40}$ or Aβ$_{1-42}$. Colchicine uptake was calculated as percent control relative to that measured in the absence of Aβ, and the peptide concentration causing 50% inhibition of uptake, D_m, was estimated using the median effect equation (DiDiodato and Sharom 1997).

Direct transport of β-amyloid peptide across mdr1 membrane vesicles

Inside-out membrane vesicles were prepared from the wild-type AuxB1 CHO cell line (CH^{R}AuxB1) and its colchicine-selected mdr1 over-expressing progeny B30 CHO cells (CH^{R}B30) as described (Juliano and Ling 1976; Shapiro and Ling 1995). To allow for Aβ incorporation into the vesicle membrane, 100 nM of either synthetic human Aβ$_{1-40}$ or Aβ$_{1-42}$ (RBI) was added to a 70-μL suspension of vesicles and allowed to equilibrate at 37°C for 15 min. Unincorporated Aβ was excluded by passage of the vesicles through a BioGel P-6 size exclusion column (BioRad, Mississaugha, Ontario). To activate transport, Na_4ATP (Sigma) at a final concentration of 1.5 mM was added to the vesicles and the reaction allowed to proceed for 15 min at 37°C. Vesicles were then ruptured using five cycles of rapid freeze-thaw in liquid nitrogen and subjected to ultracentrifugation at 100 000 *g* for 20 min. The supernatant containing intravesicular Aβ was harvested and TCA precipitated as described above for Aβ detection and the pellet containing membrane-bound Aβ was directly resuspended in Laemmli buffer. Both membrane-bound and intravesicular Aβ were subjected to western blot analysis using the 6E10 antibody as described above. Each trial (*n*) represents individual experiments performed on fresh aliquots of reconstituted membranes on separate occasions.

Quantification and statistical analysis of results

For quantification of western blots, densitometry of detected bands was performed using a molecular dynamics image quantifier. Densitometric measurements were performed in the linear range as determined by standard dilution curves of secreted cellular proteins. Optical density values are reported as percentage control. Each trial (*n*) represents individual experiments performed on different cells plated separately and completely repeated on at least three separate occasions. Analysis of variance followed by a Dunnett's *post hoc* analysis was used to determine the significance of observed differences.

Results

In order to study the cellular interaction between Aβ and MDR1, we transiently transfected pHaMDR1/A into HEK293 cells which were stably transfected with APP695 harbouring the Swedish double mutation (K269 cells) using the calcium phosphate method. In cells that were transfected with MDR1, we observed an increase in Aβ compared with mock and untransfected controls (Fig. 1a). We repeated this experiment in a slightly different paradigm by cotransfecting pHaMDR1/A and pCDN3.1APP695sw (encoding the Swedish double mutation of APP695) into wild-type HEK293 cells using the gentler Lipofectamine method of transfection. Aβ secretion increased in HEK293 cells

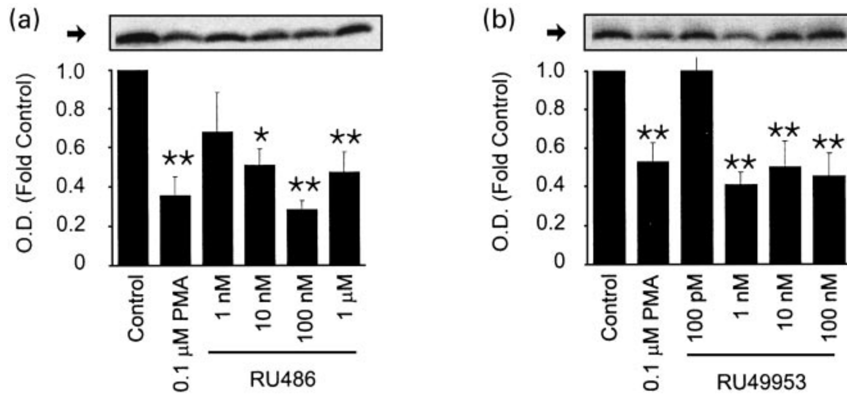

Fig. 2 Inhibition of p-gp reduces Aβ secretion. K269sw cells were transiently transfected with human *MDR1* as in Fig. 1 and exposed to (a) RU486 or (b) RU49953, at varying doses for 15 min. Both RU486 ($n = 6$) and RU49953 ($n = 6$) significantly decreased Aβ secretion in these cells in a dose-dependent manner (*$p < 0.05$, **$p < 0.01$). Aβ was detected using the WO-2 monoclonal antibody.

cotransfected with MDR1 and APP695sw (Fig. 1b). Transfection of MDR1 did not result in significant increases in expression of MDR1 protein as detected by antibody C219, but cellular levels of APP were consistently increased in association with the increase in extracellular Aβ levels (Fig. 1c). Neither the increase in Aβ secretion nor the increase in cellular APP levels was observed in cells that were transfected with MRP1, an ABC transporter with very low homology to MDR1 (Figs 1b and c). Several plausible explanations for the increased expression of APP following transfection with pHaMDR1/A include increased APP expression in response to elevated extracellular levels of Aβ, enhanced transport of an (unknown) endogenous substrate of MDR1 which alters APP expression, and non-specific effects of increased expression of an integral membrane protein. As a result of these changes in APP expression, it was impossible to determine whether the observed increases in extracellular Aβ after transfection of pHaMDR1/A were due to increases in Aβ secretion or the availability of additional substrate in the form of increased cell-associated APP. For these reasons, we turned to other methods to further address the hypothesis that p-gp might be an Aβ efflux pump.

Pharmacological treatment of K269sw cells transiently transfected with pHaMDR1/A with the MDR1 inhibitors RU486 (Gruol *et al.* 1994) and RU49953 (Marsaud *et al.*

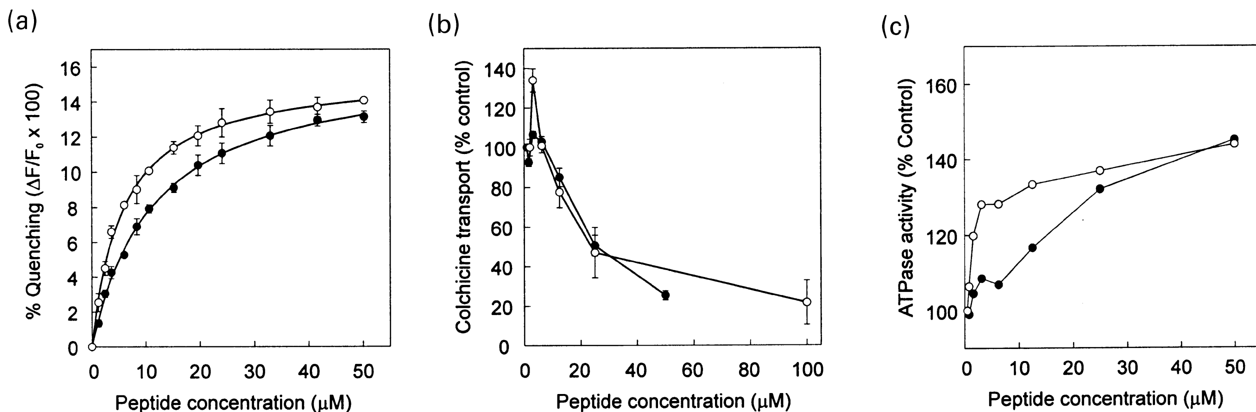

Fig. 3 (a) Binding of Aβ peptides to MIANS-labeled p-gp results in fluorescence quenching. Highly purified MIANS-labeled p-gp (50 μg/mL) was titrated with increasing concentrations of peptides $A\beta_{1-40}$ (●) and $A\beta_{1-42}$ (○). The percent quenching of the fluorescence emission at 420 nm ($\Delta F/F_0 \times 100$) was calculated relative to MIANS-labeled p-gp in the absence of peptides. The quenching data (shown by the symbols, means ± range, $n = 2$) were fitted to an equation describing interaction of the peptides with a single binding site, as indicated by the continuous line. (b) Aβ peptides block p-gp-mediated drug transport. Equilibrium uptake of [^{3}H]-colchicine into CH^RB30 plasma membrane vesicles was determined at 22°C in the presence of 1 mM ATP and a regenerating system, and increasing concentrations of Aβ peptides. Data are presented as percent of control ATP-dependent [^{3}H]-colchicine uptake in the absence of peptide (means ± SEM, $n = 3$). (c) Aβ peptides stimulate p-gp ATPase activity. CH^R B30 plasma membrane vesicles were assayed for Mg^{2+}-dependent ATPase activity in the presence of increasing concentrations of $A\beta_{1-40}$ (●) and $A\beta_{1-42}$ (○). Data are presented as a percentage of control ATPase activity measured in the absence of peptides (means ± SEM, $n = 3$). Where error bars are not visible, they are contained within the symbols.

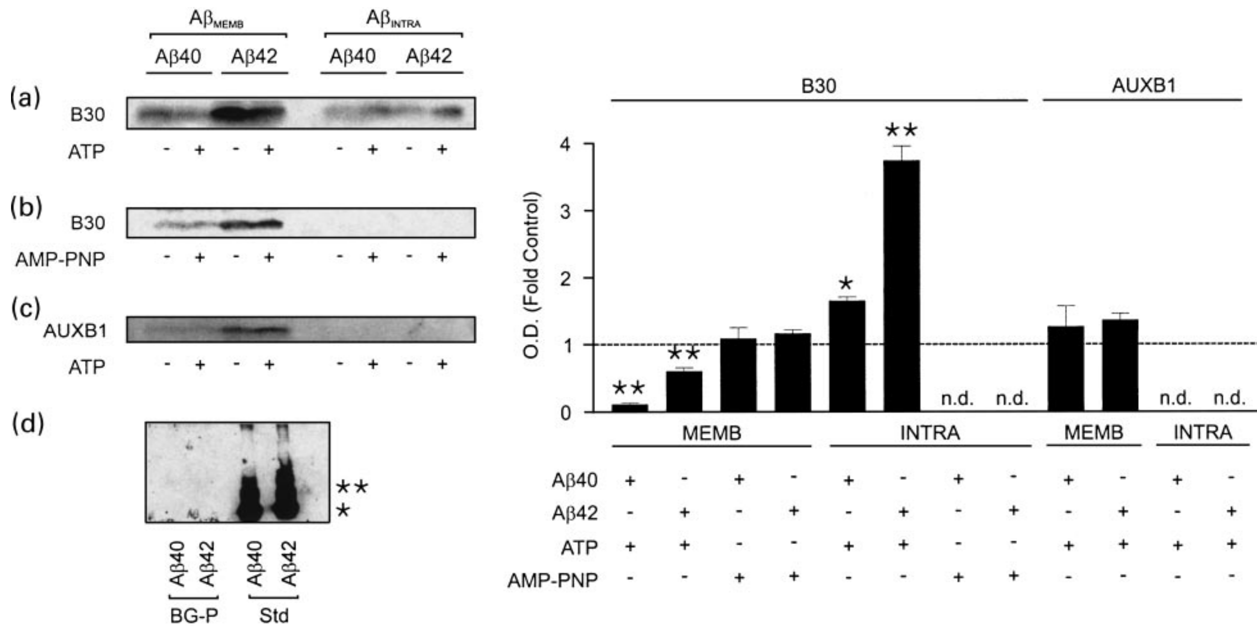

Fig. 4 p-Glycoprotein mediates transport of Aβ peptides in an ATP-dependent manner. (a) B30 vesicles enriched in hamster class I p-gp transports preinserted synthetic human $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides in an ATP-dependent manner ($n = 3$). In this and subsequent panels, western blots using the 6E10 antibody show levels of membrane-bound Aβ peptides ($A\beta_{MEMB}$) and their corresponding levels in the interior of the vesicle ($A\beta_{INTRA}$) before and after addition of nucleotide. (b) The non-hydrolysable ATP analog, AMP-PNP, does not stimulate transport of Aβ into B30 vesicles ($n = 2$). (c) ATP-dependent transport is also absent in p-gp deficient AuxB1 vesicles ($n = 3$). No Aβ was detectable within B30 and AuxB1 vesicles treated with AMP-PNP or ATP, respectively. (d) Overexposed western blot of synthetic Aβ peptides spun through a Biogel-P6 size exclusion column (BG-P) compared with standards (Std). Aβ standards (100 nM) develop an intense signal while eluant collected from solution containing 100 nM Aβ spun through BioGel-P6 columns show no detectable signal even after overexposure of the blot to ECL film, showing complete binding of Aβ by the column. Single asterisk indicates monomeric Aβ at ~4 kDa; a double asterisk indicates Aβ dimers at ~8 kDa. Aβ was detected using the W0–2 monoclonal antibody. (e) Quantification of direct transport assay results. Average O.D. values are normalized to their respective controls (dashed line, $*p < 0.05$ and $**p < 0.01$). MEMB represents membrane-bound Aβ, INTRA represents Aβ in the vesicle interior, and n.d. represents non-detectable Aβ signal. Results are expressed as mean ± SEM.

1998) significantly decreased Aβ secretion compared with cells treated with vehicle after a 15 minute drug exposure (Figs 2a and b); the 15-min time frame was chosen to avoid any possible effects of RU486 upon gene expression via glucocorticoid or progesterone receptors (Wehling 1994). In these experiments, phorbol ester (PMA) was utilized as a positive control, as it is well known to decrease Aβ secretion through activation of second messenger cascades (Mills and Reiner 1999). RU486 significantly decreased Aβ secretion from control with an EC_{50} of ~10 nM (Fig. 2a); the drug exhibited U-shaped pharmacokinetics, with maximal inhibition decreasing as the concentration of drug was brought into the micromolar range. RU49953, an analog of RU486 which acts as an MDR1 inhibitor but does not show apparent binding to steroid hormone receptors (Marsaud *et al.* 1998), decreased Aβ secretion with an EC50 of ~ 1 nM (Fig. 2b).

We next tested the hypothesis that Aβ might bind p-gp *in vitro* using highly purified hamster mdr1 reconstituted into vesicles. Binding of a wide variety of mdr1 substrates, including drugs, modulators, and cyclic and linear peptides, can be quantified by fluorescence quenching of highly purified protein labeled with the fluorophore MIANS at two conserved Cys residues within the Walker A motifs of the protein's nucleotide binding domains (Sharom *et al.* 1998a). Titration of MIANS-labeled mdr1 with synthetic peptides encoding either human $A\beta_{1-40}$ or $A\beta_{1-42}$ resulted in saturable quenching of MIANS fluorescence, suggesting that both peptides interact directly with the transporter (Fig. 3a). The binding affinities (K_d) as determined from two independent quenching titrations on different batches of mdr1 were 12.5 ± 1.0 μM and 6.7 ± 1.0 μM for $A\beta_{1-40}$ and $A\beta_{1-42}$, respectively. Precedent for binding (Sharom *et al.* 1998a) and secretion (Sharom *et al.* 1996) of peptides by mdr1 exists, with most peptide substrates having K_d values similar to that exhibited by Aβ.

Bona fide mdr1 substrates are generally capable of competing for transport with other substrates in both plasma membrane and proteoliposome systems (Doige and Sharom 1992; Sharom *et al.* 1993). To test the hypothesis that Aβ is capable of competing with established mdr1 substrates, we

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tested the ability of Aβ to alter ATP-dependent uptake of [^{3}H]-colchicine into plasma membrane vesicles derived from colchicine selected, mdr1 overexpressing Chinese hamster ovary CH^{R}B30 cells. Both Aβ_{1-40} and Aβ_{1-42} competed effectively with [^{3}H]-colchicine for transport, with the concentration required for 50% inhibition of drug uptake, D_m, estimated to be 27 μM for Aβ_{1-40}, and 22 μM for Aβ_{1-42} (Fig. 3b).

The ability of mdr1 to transport substrates is dependent upon hydrolysis of ATP, and substrates for transport often stimulate ATPase activity. To test the hypothesis that Aβ peptides might stimulate mdr1 ATPase activity, Aβ_{1-40} and Aβ_{1-42} were added to plasma membrane vesicles derived from CH^{R}B30 cells and the resultant ATPase activity measured (Fig. 3c). Both Aβ_{1-40} and Aβ_{1-42} stimulated ATPase activity; Aβ_{1-40} increased ATPase activity by 100% at a concentration of 50 μM (half-maximal stimulation at 17 μM), whereas in the case of Aβ_{1-42}, stimulation of ~40% was observed at 50 μM (half-maximal stimulation at 2 μM). Taken together, these data define Aβ as a *bona fide* mdr1 substrate.

To directly test the hypothesis that an ABC transporter can transport Aβ, we developed an *in vitro* assay in which Aβ transport across the membrane could be directly measured. For these experiments, we used vesicles prepared from CH^{R}B30 cells (Juliano and Ling 1976; Shapiro and Ling 1995). During reconstitution of these vesicles, mdr1 proteins are incorporated in both the normal configuration and in an inside-out configuration; addition of ATP to the external medium selectively activates mdr1 oriented in the inside-out orientation with its ATP binding sites on the outside of the vesicle, thus allowing for transport of substrates from the outside to the lumen of the vesicles. In order to reconstruct the physiological association of Aβ with the membrane, we incorporated synthetic human Aβ peptides into these vesicles. Since the sequence of human and rodent Aβ differs, antibodies specific to human Aβ selectively measure transport of the synthetic human Aβ across these membranes and prevents detection of endogenous rodent Aβ in the vesicle membrane. Vesicles were incubated with either Aβ_{1-40} or Aβ_{1-42} (100 nM) for 15 min at 37°C and any free unbound Aβ in the solution was removed by passage through a size-exclusion column (Fig. 4d). ATP was then added to the solution, incubated at 37°C for 15 min to activate mdr1 and the membrane and intravesicular fractions were separated and Aβ levels measured using western blot analysis.

We observed a significant decrease in membrane-bound Aβ with a corresponding increase in intravesicular Aβ (Figs 4a and e). In contrast, vesicles treated with the non-hydrolysable ATP analog AMP-PNP showed no significant changes in either membrane-bound or lumenal Aβ (Fig. 4b and e), demonstrating that transport of Aβ is energy dependent. Transport was also dependent upon overexpression of mdr1, as no detectable changes in Aβ content were observed in either the membrane or the intravesicular compartment when the experiment was carried out using vesicles prepared from the parental AuxB1 cells which are not enriched in hamster mdr1 (Figs 4c and e). Taken together, these data provide strong evidence that mdr1 is an Aβ transporter.

Discussion

The events involved in the production of Aβ are increasingly being understood. The process begins with cleavage of APP by the recently identified enzyme β-secretase (Vasser *et al*. 1999; Lin *et al*. 2000), yielding an extracellular fragment known as sAPPβ which is simply shed into the extracellular space (Mills and Reiner 1999). The remaining 99 amino acid COOH-terminal fragment (C99) consists of 28 charged amino acids on the extracellular side of the membrane, 23 hydrophobic amino acids which presumably traverse the membrane as an α-helix, and 52 charged amino acids constituting the intracellular domain of the polypeptide. The Aβ peptide is produced following cleavage of C99 *within* the membrane (Brown *et al*. 2000) by an enzyme known as γ-secretase [which appears to be identical to the presenilins (Wolfe *et al*. 1999; Lin *et al*. 2000)]. The resulting 40 and 42 amino acid versions of Aβ are amphipathic, consisting of 28 charged amino acids and either 12 or 14 hydrophobic amino acids (for Aβ_{1-40} and Aβ_{1-42}, respectively). The hydrophobic nature of Aβ is consistent with data indicating that the peptide has limited solubility in aqueous solutions (Terzi *et al*. 1995) with a preference for electrostatic binding to the membrane bilayer (Terzi *et al*. 1997). These observations suggest that constitutive release of Aβ from cells may be an active process, and our data demonstrating that Aβ secretion can occur through MDR1 leads us to propose that ABC transporters can act as Aβ efflux pumps.

How might an ABC transporter such as MDR1 act as an Aβ efflux pump? One model is based upon the so-called vacuum-cleaner hypothesis (Gottesman and Pastan 1993), in which the ABC transporter draws the Aβ peptide laterally from within the membrane and moves it from the energetically favorable environment of the lipid bilayer into the aqueous environment of the extracellular space. A related model involves the transporter acting as a flippase (Higgins and Gottesman 1992), either moving the peptide from the inner to the outer leaflet of the membrane or locally altering membrane lipid composition such that the peptide detaches. These observations are not only relevant to the molecular basis of Aβ secretion, they may also be applicable to the mechanism by which amphipathic peptides, proteins lacking signal sequences, or lipid-modified proteins detach from biological membranes (Kuchler and Thorner 1992; Ambudkar *et al*. 1999; Yakushi *et al*. 2000).

Two aspects of our findings are of relevance to AD. The first is that Aβ is unlikely to aggregate while attached to the membrane, as the hydrophobic amino acids in the COOH tail of the peptide would be shielded by their association with the lipid bilayer. Thus, detachment of Aβ from the membrane represents a critical change in the biophysical properties of Aβ, and is likely to be a prerequisite to the aggregation events which are thought to be at the core of the pathology. The second observation of merit is that MDR1 is expressed at high levels at the lumenal surface of cerbrovascular endothelial cells (Cordon-Cardo *et al*. 1989), and perhaps at the end-feet processes of astrocytes (Pardridge *et al*. 1997). Thus, one could speculate that changes in MDR1 function and/or expression might alter the clearance of Aβ from within the brain, and may even contribute to cerebrovascular amyloid angiopathy. In this regard, it is interesting to note that MDR1 function can be modified in the absence of changes in expression, as has recently been demonstrated by examining the effects of dexamethasone upon vincristine transport in endothelial cells *in vitro* (Regina *et al*. 1999). Of greater importance to the development of Alzheimer therapeutics is the observation that cells throughout the body constitutively produce and release Aβ, yet the MDR1 protein is only expressed in a limited number of tissues, and is essentially undetectable in neurons (Fojo *et al*. 1987; Thiebaut *et al*. 1987). Given the substrate promiscuity between members of the ABC transporter superfamily (Ford and Hait 1990), it is likely that other brain-expressed ABC transporters are capable of sustaining Aβ efflux. Identifying such neuronal Aβ efflux pumps may open new avenues for ameliorating the Aβ burden in the Alzheimer brain.

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