Published in final edited form as:

Neuroscience. 2003 ; 121(2): 487–492.

Efflux of Human and Mouse Amyloid β Proteins 1-40 and 1-42 From Brain: Impairment in a Mouse Model of Alzheimer's Disease

William A. Banks, Sandra M. Robinson, Sulekha Verma, and John E. Morley

GRECC, Veterans Affairs Medical Center-St. Louis and Division of Geriatrics, Department of Internal Medicine, Saint Louis University School of Medicine

Abstract

Brain to blood transport is believed to be a major determinant of the amount of amyloid β protein (A β P) found in brain. Impaired efflux has been suggested as a mechanism by which A β P can accumulate in the CNS and so lead to Alzheimer's disease (AD). To date, however, no study of the efflux of the form of A β P most relevant to AD, A β P1-42, has been conducted, even though a single amino acid substitution in A β P can greatly alter efflux. Here, we examined the efflux of A β P mouse1-42, mouse1-40, human1-42, and human1-40 in young CD-1, young SAMP8, and aged SAMP8 mice. The SAMP8 mouse with aging spontaneously overproduces A β P and develops cognitive impairments reversed by A β P-directed antibody or phosphorothioate antisense oligonucleotide. CD-1 mice transported all forms of A β P, although mouse1-42 and human1-40 were transporter faster than the other forms. There was a decrease in the saturable transport of mouse1-42 in SAMP8 mice regardless of age. Efflux of mouse1-40 and human1-42 was only by a non-saturable mechanism in young SAMP8 mice and their efflux was totally absent in aged SAMP8 mice. These differences in the efflux of the various forms of A β P among the three groups of mice supports the hypothesis that impaired efflux is an important factor in the accumulation of A β P in the CNS.

Keywords

Blood-brain barrier; dementia; peptide; central nervous system; SAMP8; cognition

Introduction

Amyloid beta protein (A β P) is thought to play a causal role in the development of Alzheimer's disease (Sambamurti et al., 2002) (Banks and Morley, 2003; Rosenberg, 2000). A β P is variably cleaved at the C-terminus to form peptides 39-43 amino acids in length (Rosenberg, 2000; Selkoe, 1990). The 1-40 and 1-42 peptides are the most common and the most widely studied. In general, A β P1-40 is less neurotoxic, less common in the neuritic plaques of AD, and less likely to be involved in the neuropathology of AD than A β P1-42. However, A β P1-42 is more difficult to study than A β P1-40 because of polymerization.

As is the case for any peptide, the levels of A β P are a balance between its rates of synthesis and degradation (Sambamurti et al., 2002). Both of these rates have been postulated to be altered in AD. Additionally, the level of A β P in the CNS is affected by its rate of clearance

from the brain (Banks et al., 1997b; DeMattos et al., 2002; Ghersi-Egea et al., 1996; Shibata et al., 2000).

A β P is cleared from the brain by two major mechanisms. First, A β P in the cerebrospinal fluid is transferred to blood across arachnoid villi and lymphatic drainage along with reabsorption of the CSF. This is a passive mechanism and affects all substances found in the CSF. Second, A β P1-40 has been shown to be transported across brain capillaries by a saturable efflux system (Bading et al., 2002; DeMattos et al., 2002; Ghersi-Egea et al., 1996; Monroe et al., 2002; Shibata et al., 2000). This transporter, tentatively identified as LDL receptor-related protein-1 (Shibata et al., 2000), has been suggested to be impaired in AD (Bading et al., 2002; Banks et al., 1999; Ghersi-Egea et al., 1996; Rosenberg, 2000). Such impairment could be a factor in the accumulation of A β P in the CNS and the onset of AD. Improved efflux may be one mechanism by which antibody directed against A β P can reverse CNS loads of A β P and lead to cognitive improvement in mice overexpressing amyloid precursor protein (Bacskai et al., 2001; Banks et al., 2002; Bard et al., 2000; DeMattos et al., 2002; Zlokovic et al., 2000).

However, the efflux studies have only been performed with the human A β P1-40, mostly in rodent models. Blood-brain barrier (BBB) transport systems can exhibit species specificity. For example, human interleukin-1 γ is transported across the mouse, but not the rat, BBB (Plotkin et al., 2000). Additionally, minor changes in peptide primary structure can alter peptide transport rate. For example, Tyr-MIF-1 is transported from brain to blood but not blood to brain, whereas MIF-1, which differs from Tyr-MIF-1 by only an N-terminal tyrosine, is transported into, but not out of, the CNS (Banks and Kastin, 1994). Some species of A β P also show such specificity. A single amino acid substitution at position 22 from glutamate to glycine reduces CNS to blood efflux of A β P1-40 by 7 fold (Monroe et al., 2002). Furthermore, most of the residual transport occurred at the choroid plexus and not at the vascular BBB. Mouse1-42 and human1-40 differ by 5 amino acids (table 1).

The above raises two fundamental questions which we address here. The first question is whether A β P1-42 is transported out of the brain. A β P1-42 is considered the most neurotoxic of the A β P's and should be the peptide of greatest interest in BBB studies. However, to date, only the efflux of the A β P1-40 has been investigated. The second question is whether any measurable efflux of A β P1-42 is impaired in a mouse model of spontaneous overproduction of A β P. The mouse strain (SAMP8) investigated has a natural mutation which leads to age-related overexpression of APP, accumulation of A β P, and cognitive defects (Flood et al., 1993; Flood and Morley, 1998; Morley et al., 2000; Morley et al., 2002; Nomura et al., 1996). The learning and memory deficits in SAMP8 mice are reversed by administration of A β P directed antibodies and antisense to APP mRNA (Banks et al., 2001; Kumar et al., 2000; Morley et al., 2000; Morley et al., 2002). A subset of this question is whether any such impairments in efflux would precede or follow the onset of A β P accumulation. If following, then the impaired efflux could be related to the increased A β P levels. If preceding, then impaired efflux could be a factor in the age-related accumulation of A β P.

Methods

Radioactive Labeling and Purification of A β P: Five μ g of one of four carrier-free, recombinant amyloid beta proteins were used: mouse1-42, mouse1-40, human1-42, human1-40 (American Peptide Co, Sunnyvale, CA) was labeled by the chloramine-T method. This was purified on a column of G-10 Sephadex by eluting 0.1 ml fractions with protein-free, chloride-free phosphate buffer solution. Both SDS and non-denaturing gels showed that about 70% of radioactively labeled mouse1-42 eluted as monomer (figure 1).

Measure of CNS to Blood Efflux Rates: The method used to quantify the fate of centrally administered I-A β P has been described previously (Banks and Kastin, 1989) and has been used to assess brain efflux (Banks et al., 1986; Banks et al., 1990) and sequestration (Banks and Broadwell, 1994; Cashion et al., 1996). Two month old male ICR mice, two month old SAMP8 mice, or twelve month old SAMP8 mice (all from our in-house colonies) were kept on a 12/12 hour light/dark cycle with food and water freely available. They were anesthetized on day of study with 0.15 ml of 40% urethane. The scalp was removed and a hole made into the lateral ventricle, 1.0 mm lateral and 1.0 mm posterior to the bregma, with a 26 gauge needle with a tubing guard which kept the depth of the holes constant (3.0-3.5 mm). Mice received 1.0 μ l intracerebroventricular (icv) injections containing 5,000 cpm (about 0.125 ng) of I-A β P in a lactated Ringer's solution with 1% bovine serum albumin. Mice were decapitated at 2, 5, 10, and 20 minutes after injection. The whole brain was removed, the pituitary and pineal were discarded, and the level of residual radioactivity in the whole brain was determined from the counts after 3 minutes in a gamma counter. The level of radioactivity in whole brain at t=0 was determined in mice overdosed with anesthetic as previously described (Banks and Kastin, 1989). The cpm remaining in the brain was divided by the cpm injected and multiplied by 100 to yield the percent of the injected dose remaining in brain (%Inj/brain). The log of this value was plotted against time, and the half-time disappearance was calculated by multiplication of the inverse of the slope of this relationship by 0.301. Regression lines were compared with the Prism 3.0 program and as described below under Statistical Analysis.

The possible presence of a saturable component was tested by giving another group of mice an icv injection of I-A β P with or without 1 μ g/mouse of unlabeled A β P included in the injection. These mice were decapitated 10 min after the icv injection, the means and error terms calculated, and the two groups compared by t-test.

Statistical Analysis: Means are reported with the number of mice used (n) and the standard error of the mean (SEM). Student's t-test was used for comparison of two groups. More than two groups were compared by analysis of variance (ANOVA) followed by Newman-Keuls post test. The p values were reported for relevant statistically significant differences. Regression lines were calculated by the least squares method with the Prism 3.0 program (GraphPad, Inc., San Diego, CA) and the slope (m) with its standard deviation of the mean, the intercept (i) with its error term, the correlation coefficient (r), the number of points on which the line was based (n) and the p value reported. Regression lines were compared for statistical differences with the Prism 3.0 program, which first determines whether there are differences between slopes and, if not, whether there are differences between intercepts. More than two slopes were compared by ANOVA followed by Newman-Keuls range test with the standard deviation of the mean taken as the standard error term and, because two means (the slope and the intercept) were calculated from the data, n-1 was used as the value for n.

Results

Efflux of A β P Mouse1-42: The relation between log(%Inj/brain) and time was statistically significant for the CD-1 ($m = -0.0190 \pm 0.0020$, $i = 1.58 \pm 0.020$, $r = 0.959$, $n = 10$, $p < 0.001$), young SAMP8 ($m = -0.0079 \pm 0.0025$, $i = 1.54 \pm 0.026$, $r = 0.773$, $n = 9$, $p < 0.05$), and aged SAMP8 groups ($m = -0.0054 \pm 0.0016$, $i = 1.54 \pm 0.017$, $r = 0.792$, $n = 9$, $p < 0.05$), demonstrating a measurable efflux of mouse1-42 in all three groups of mice (Figure 2, upper panel). However, statistical comparison of the lines showed that differences existed among the slopes of the line: $F(2,64) = 7.87$, $p < 0.001$. ANOVA confirmed a difference among slopes [$F(2,24) = 12.7$, $p < 0.001$]. Newman-Keuls range test showed that the slope of the line for CD-1 mice was different from the slope of the line for the young

SAMP8 ($p < 0.001$) and aged SAMP8 mice ($p < 0.001$). There was no difference between the SAMP8 young and aged mice (Figure 2, bottom panel) even by *t*-test. The half-time disappearances calculated from the slopes were 15.8 min for the CD-1 mice, 38.0 min for the young SAMP8 mice, and 55.9 min for the aged SAMP8 mice. Coinjection of unlabeled mouse1-42 (1 :g/mouse) with the radioactive mouse1-42 showed a saturable component to efflux in all three groups of mice (Figure 3).

Efflux of Other A β P in CD-1 mice: Each of the other A β P's had a significant relation between their $\log(\% \text{Inj}/\text{brain})$ and time, demonstrating that the CD-1 mouse could clear each of them: mouse1-40: ($m = -0.0076 \pm 0.0031$, $i = 1.56 \pm 0.032$, $r = 0.660$, $n = 10$, $p < 0.05$); human1-42: ($m = -0.0076 \pm 0.0024$, $i = 1.57 \pm 0.025$, $r = 0.739$, $n = 10$, $p < 0.05$); human1-40: ($m = -0.0185 \pm 0.0026$, $i = 1.51 \pm 0.026$, $r = 0.931$, $n = 10$, $p < 0.001$). This yielded half-time disappearance rates of 39.4 min (mouse1-40), 39.7 min (human1-42), and 16.3 min (human1-40). Comparison of the slopes showed a statistical difference by 2-way ANOVA with mouse group (CD-1, young SAMP8, aged SAMP8) and peptide (mouse1-42, mouse1-40, human1-42, human1-40) being the two independent variables for interaction ($p < 0.001$), a trend for peptide ($p = 0.07$) and no effect for mouse group. The Newman-Keuls range test showed that the efflux by CD-1 mice of mouse1-42 and human1-40 was faster than the efflux of mouse1-40 and human1-42 (Figure 4). Efflux of each of these peptides was inhibited by its unlabeled version, showing each was transported out of the CNS by a saturable system (Table 2). The ANOVA showed that each of the A β P were able to cross-inhibit the efflux of radioactive mouse1-42 out of the brains of CD-1 mice: $F(3,21) = 20.2$, $p < 0.001$ (Figure 5). Newman-Keuls range test showed that each peptide inhibited at the $p < 0.001$ level with no difference among the peptide groups.

Efflux of Mouse1-40, Human1-42, and Human1-40 in SAMP8: A significant relation between $\log(\% \text{Inj}/\text{brain})$ and time existed for mouse1-40 ($m = -0.0047 \pm 0.0019$, $i = 1.62 \pm 0.020$, $r = 0.658$, $n = 10$, $p < 0.05$) and human1-42 ($m = -0.0069 \pm 0.0026$, $i = 1.59 \pm 0.027$, $r = 0.683$, $n = 10$, $p < 0.05$) in young SAMP8 mice, demonstrating efflux. The half-time disappearance was 63.5 min for mouse1-40 and 43.3 min for human 1-42. However, 1 :g/mouse of unlabeled peptide co-injected with its radioactive version did not affect the cpm retained by brain, showing that there was not a saturable component to efflux (Table 2).

A significant relation between $\log(\% \text{Inj}/\text{brain})$ and time did not exist for mouse1-40 or human1-42 in aged SAMP8 mice, demonstrating the absence of efflux. Consistent with this, 1 :g/mouse of unlabeled peptide co-injected with its radioactive version did not affect the cpm retained by brain.

Human1-40 had a significant relation between $\log(\% \text{Inj}/\text{brain})$ and time in both young ($m = -0.0047 \pm 0.0018$, $i = 1.62 \pm 0.018$, $r = 0.683$, $n = 10$, $p < 0.05$) and aged SAMP8 mice ($m = -0.0139 \pm 0.0019$, $i = 1.66 \pm 0.020$, $r = 0.931$, $n = 10$, $p < 0.001$). The slopes yielded half-time disappearance rates of 64.6 min (young) and 21.6 min (aged) mice. Unlabeled human1-40 inhibited the efflux of radioactive human1-40 in both young ($p < 0.001$) and aged ($p < 0.05$) mice.

Discussion

Several original observations arose from this first study to examine the CNS to blood efflux of A β P1-42 in vivo. We found that mouse1-42 was transported out of the CNS by a saturable transport system. All three groups of mice studied (young CD-1, young SAMP8, and aged SAMP8) were able to transport A β P1-42 out of the CNS by a saturable mechanism. However, the SAMP8 strain regardless of age was much less efficient in transporting A β P out of the CNS. We also found that CD-1 mice transported other forms of

A β P at varying rates. Species specificity existed, with mouse1-42 transported much more quickly than mouse1-40 or human1-42. Evidence suggests that a single transporter or a family of transporters with overlapping affinities are responsible for the efflux of the A β P. However, the SAMP8 transport system is less universal and is shifted in its efficiency of transport away from mouse1-42 towards human1-40.

Mouse1-42 was transported out of the CNS by all three groups of mice by a saturable mechanism. The method used to examine efflux has several advantages over other efflux methods (Banks et al., 1997a; Banks and Kastin, 1989). First, it can use mice, as opposed to rats, sheep, or larger animals. Second, the icv injection version used here, as opposed to the brain parenchymal injection version or other brain parenchymal injection methods, measures global efflux; that is, it measures efflux at both the vascular and choroid plexus barriers. Finally, it quantifies actual efflux as opposed to producing an index or ratio relative to a reference substance. Therefore, the method can be used in systems where underlying assumptions of bulk flow or CNS distribution may not hold.

Efflux of mouse1-42 varied between the CD-1 and SAMP8 strains of mice. CD-1 mice were much more efficient at transporting mouse1-42 than either young or aged SAMP8 mice. The CD-1 also transported by a saturable system each of the other 3 A β P's. Transport in CD-1 was faster for mouse1-42 and human1-40 than for mouse1-40 or human1-42. The reason why CD-1 mice transport the two most different peptides equally well is unclear. If a single transporter exists for all forms of A β P, then it may be that these two forms are most similar in a critical tertiary structure important to A β P efflux. Alternatively, there may be more than one efflux system for A β P.

Although SAMP8 mice could also transport mouse1-42 by a saturable system, they did so at a slower rate. Both young and aged SAMP8 mice were equally impaired, showing that the transporter deficiency predates the accumulation of A β P and plaque formation found in the aged, cognitively impaired SAMP8. This suggests that impaired efflux could be an early contributor to accumulation of A β P in the CNS. This clearly supports the idea that a similar process in humans could be critical to the development of AD.

Efflux of the other forms of A β P were also affected in the SAMP8 mouse. The efflux of mouse1-40 and human1-42 in young CD-1 had no saturable component. Non-saturable reabsorption of CSF occurs at the arachnoid villi and the primitive lymphatics of the brain. Any substance dissolved in the CSF will exit the CNS by these mechanisms. Human1-40, although transported out of the CNS by a saturable system in the SAMP8, was effluxed at a slower rate in the SAMP8 mouse. These findings support the above observations for mouse1-42 and again suggest that impaired brain efflux may be a contributing factor to the accumulation of A β P in the SAMP8.

An age effect was observed for the transport of A β P. No statistically significant efflux, either saturable or non-saturable, could be found for mouse1-40 or human1-42. Given that any substance remaining in the CSF should have a measurable, non-saturable efflux, this suggests that the aged SAMP8 somehow disposes of these A β P's differently than the young SAMP8. One possibility is that the A β P is retained by brain parenchyma, possibly in the formation of plaques.

In conclusion, we found that the mouse1-42 version of A β P is transported out of the CNS in CD-1, young SAMP8, and aged SAMP8 mice. However variations in the efflux of A β P among these three groups of mice and among the various forms of A β P support the hypothesis that an impaired transport could be a significant contributor to A β P accumulation within the brain and to the development of AD.

Acknowledgments

Supported by VA Merit Review and R01 NS41863 and R01 AA12743.

Abbreviations

AD	Alzheimer's disease
AβP	Amyloid beta protein
ANOVA	Analysis of variance
BBB	Blood-brain barrier
icv	Intracerebroventricular
%Inj/brain	The percent of an injected dose entering a gram of brain
human1-40	Human A β P1-40
human1-42	Human A β P1-42
mouse1-40	Mouse A β P1-40
mouse1-40	Mouse A β P1-42

Reference List

- Bacskai BJ, Kajdasz ST, Christie RH, Carter C, Games D, Seubert P, Schenk D, Hyman BT. Imaging of amyloid- β deposits in brains of living mice permits direct observation of clearance of plaques with immunotherapy. *Nature Medicine*. 2001; 7:369–372.
- Bading JR, Yamada S, Mackic JB, Kirkman L, Miller C, Calero M, Ghiso J, Frangione B, Zlokovic BV. Brain clearance of Alzheimer's amyloid- β 040 in the squirrel monkey: A SPECT study in a primate model of cerebral amyloid angiopathy. *J Drug Targeting*. 2002; 10:359–368.
- Banks WA, Broadwell RD. Blood to brain and brain to blood passage of native horseradish peroxidase, wheat germ agglutinin and albumin: pharmacokinetic and morphological assessments. *J Neurochem*. 1994; 62:2404–2419. [PubMed: 7514652]
- Banks WA, DiPalma CR, Farrell CL. Impaired transport of leptin across the blood-brain barrier in obesity. *Peptides*. 1999; 20:1341–1345. [PubMed: 10612449]
- Banks WA, Farr SA, Butt W, Kumar VB, Franko MW, Morley JE. Delivery across the blood-brain barrier of antisense directed against amyloid β : reversal of learning and memory deficits in mice overexpressing amyloid precursor protein. *J Pharmacol Exp Ther*. 2001; 297:1113–1121. [PubMed: 11356936]
- Banks, WA.; Fasold, MB.; Kastin, AJ. Measurement of efflux rate from brain to blood.. In: Irvine, GB.; Williams, CH., editors. *Methods in Molecular Biology: Neuropeptides Protocols*. Humana Press; Totowa, NJ: 1997a. p. 353-360.
- Banks, WA.; Kastin, AJ. Quantifying carrier-mediated transport of peptides from the brain to the blood.. In: Conn, PM., editor. *Methods in Enzymology*. Vol. 168. Academic Press; San Diego: 1989. p. 652-660.
- Banks WA, Kastin AJ. Opposite direction of transport across the blood-brain barrier for Tyr-MIF-1 and MIF-1: comparison with morphine. *Peptides*. 1994; 15:23–29. [PubMed: 7912427]
- Banks WA, Kastin AJ, Fischman AJ, Coy DH, Strauss SL. Carrier-mediated transport of enkephalins and N-Tyr-MIF-1 across blood-brain barrier. *Am J Physiol*. 1986; 251:E477–E482. [PubMed: 2876644]
- Banks, WA.; Kastin, AJ.; Maness, LM.; Banks, MF.; Shayo, M. Interactions of - amyloids with the blood-brain barrier. de la Torre, JC.; Hachinski, V., editors. *Ann. N.Y. Acad. Sci.*; New York, NY: 1997b. p. 190-199.[826]
- Banks WA, Morley JE. Memories are made of this: recent advances in understanding cognitive impairments and dementia. *J Gerontology*. 2003

- Banks WA, Schally AV, Barrera CM, Fasold MB, Durham DA, Csernus VJ, Groot K, Kastin AJ. Permeability of the murine blood-brain barrier to some octapeptide analogs of somatostatin. *Proc Natl Acad Sci USA*. 1990; 87:6762–6766. [PubMed: 1975697]
- Banks WA, Terrell B, Farr SA, Robinson SM, Nonaka N, Morley JE. Transport of amyloid β protein antibody across the blood-brain barrier in an animal model of Alzheimer's disease. *Peptides*. 2002
- Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert P, Schenk D, Yednock T. Peripherally administered antibodies against amyloid β -peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer's disease. *Nature Medicine*. 2000; 6:916–919.
- Cashion MF, Banks WA, Kastin AJ. Sequestration of centrally administered insulin by the brain: effects of starvation, aluminum, and TNF- α . *Hormone Behav*. 1996; 30:280–286.
- DeMattos RB, Bales KR, Cummins DJ, Paul SM, Holtzman DM. Brain to plasma amyloid- β efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. *Science*. 2002; 295:2264. [PubMed: 11910111]
- Flood JF, Morley JE. Learning and memory in the SAMP8 mouse. *Neurosci Biobehav Rev*. 1998; 22:1–20. [PubMed: 9491937]
- Flood JF, Morley JE, La Reginna M. Age-related changes in the pharmacological improvement of retention in senescence accelerated mouse (SAM). *Neurobiol Aging*. 1993; 14:159–166. [PubMed: 8487919]
- Gherzi-Egea JF, Gorevic PD, Ghiso J, Frangione B, Patlak CS, Fenstermacher JD. Fate of cerebrospinal fluid-borne amyloid β -peptide: rapid clearance into blood and appreciable accumulation by cerebral arteries. *J Neurochem*. 1996; 67:880–883. [PubMed: 8764620]
- Kumar VB, Farr SA, Flood JF, Kamlesh V, Franko M, Banks WA, Morley JE. Site-directed antisense oligonucleotide decreases the expression of amyloid precursor protein and reverses deficits in learning and memory in aged SAMP8 mice. *Peptides*. 2000; 21:1769–1775. [PubMed: 11150636]
- Monro OR, Mackie JB, Yamada S, Segal MB, Ghiso J, Maurer C, Calero M, Frangione B, Zlokovic BV. Substitution at codon 22 reduces clearance of Alzheimer's amyloid-beta peptide from the cerebrospinal fluid and prevents its transport from the central nervous system into blood. *Neurobiol Aging*. 2002; 23:405–412. [PubMed: 11959403]
- Morley JE, Farr SA, Kumar VB, Banks WA. Alzheimer's disease through the eye of a mouse: Acceptance lecture for the 2001 Gayle A. Olson and Richard D. Olson prize. *Peptides*. 2002; 23:589–599. [PubMed: 11836012]
- Morley JE, Kumar VB, Bernardo AF, Farr SA, Uezu K, Tumosa N, Flood JF. β -Amyloid precursor polypeptide in SAMP8 mice affects learning and memory. *Peptides*. 2000; 21:1761–1767. [PubMed: 11150635]
- Nomura Y, Yamanaka Y, Kitamura Y, Arima T, Ohnuki T, Oomura Y, Sasaki K, Nagashima K, Ihara Y. Senescence-accelerated mouse. *Neurochemical studies on aging*. *Ann NY Acad Sci*. 1996; 786:410–418. [PubMed: 8687038]
- Plotkin SR, Banks WA, Maness LM, Kastin AJ. Differential transport of rat and human interleukin-1 α across the blood-brain barrier and blood-testis barrier in rats. *Brain Res*. 2000; 881:57–61. [PubMed: 11033093]
- Rosenberg RN. The molecular and genetic basis of AD: The end of the beginning. The 2000 Wartenberg lecture. *Neurology*. 2000; 54:2045–2054. [PubMed: 10851361]
- Sambamurti K, Greigh NH, Lahiri DK. Advances in the cellular and molecular biology of the beta-amyloid protein in Alzheimer's disease. *Neuromolecular Medicine*. 2002; 1
- Selkoe DJ. Deciphering Alzheimer's disease: the amyloid precursor protein yields new clues. *Science*. 1990; 248:1058–1060. [PubMed: 2111582]
- Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, Holtzman DM, Miller CA, Strickland DK, Ghiso J, Zlokovic BV. Clearance of Alzheimer's amyloid- β_{1-40} peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J Clin Invest*. 2000; 106:1489–1499. [PubMed: 11120756]
- Zlokovic BV, Yamada S, Holtzman D, Ghiso J, Frangione B. Clearance of amyloid β -peptide from brain: transport or metabolism? *Nature Medicine*. 2000; 6:718–719.

Figure 1.
SDS gel of mouse ABP1-42 radioactively labeled with NaI by the chloramine-T method and purified on a column of sephadex G-10.

Figure 2.

Efflux of Aβ mouse1-42 from the CNS to Blood in CD-1, Young SAMP8, and Aged SAMP8 mice. Upper Panel: The relation between log(%Inj/brain) and time was statistically significant for each group of mice, demonstrating that Aβ was cleared from the CNS. Lower Panel: the CD-1 mice cleared mouse1-42 more quickly than the SAMP8 mice.

Figure 3.

Saturable Component to the Efflux of AβP mouse1-42 in CD-1, Young SAMP8, and Aged SAMP8 mice. All three groups of mice had a saturable component to efflux.

Figure 4.

Slopes of All AβP peptides in Each Group of Mice. A 2-way ANOVA showed statistical significance among the slopes for interaction ($p<0.001$), a trend for peptide, and no effect for mouse group. Newman-Keuls range test found the statistical differences shown: * $p<0.05$; ** $p<0.01$.

Figure 5. Effects of Various AβP's on the Efflux of radioactive Mouse1-42. Each of the peptides produced a statistically significant inhibition in the efflux of mouse1-42.

Table 1Differences Among the Species of A β P Tested.

	5	10	13	41	42
Mouse1-42	-----Gly-----	Phe----	Arg-----	Ile-Ala	
Mouse1-40	-----Gly-----	Phe----	Arg-----		
Human1-42	-----Arg-----	Tyr----	His-----	Ile-Ala	
Human1-40	-----Arg-----	Tyr----	His-----		

Not shown: Mouse and rat A β P1-42 have identical primary sequences.

Table 2Saturable Component to the CNS to Blood Efflux of A β P.

A β P	Mouse1-42	Mouse1-40	Human1-42	Human 1-40
CD-1	Sat	Sat	Sat	Sat
SAM-Young	Sat	Non-Sat	Non-Sat	Sat
SAM-Aged	Sat	Non-Sat	Non-Sat	Sat

Mice received an icv injection of a radioactive A β P with or without 1 :g/mouse of the nonradioactive version of the A β P included in the icv injection. An statistically significant ($p<0.05$) increase in the amount of radioactive A β P retained by the brain was taken as evidence of a saturable component to efflux. Sat = saturable component; Non-Sat = no saturable component found.