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Neurobiology of Disease

Acute γ-Secretase Inhibition of Nonhuman Primate CNS Shifts Amyloid Precursor Protein (APP) Metabolism from Amyloid-β Production to Alternative APP Fragments without Amyloid-β Rebound


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The accumulation of amyloid β (Aβ) in Alzheimer’s disease is caused by an imbalance of production and clearance, which leads to increased soluble Aβ species and extracellular plaque formation in the brain. Multiple Aβ-lowering therapies are currently in development: an important goal is to characterize the molecular mechanisms of action and effects on physiological processing of Aβ, as well as other amyloid precursor protein (APP) metabolites, in models which approximate human Aβ physiology. To this end, we report the translation of the human in vivo stable-isotope-labeling kinetics (SILK) method to a rhesus monkey cisterna magna ported (CMP) nonhuman primate model, and use the model to test the mechanisms of action of a γ-secretase inhibitor (GSI). A major concern of inhibiting the enzymes which produce Aβ (β- and γ-secretase) is that precursors of Aβ may accumulate and cause a rapid increase in Aβ production when enzyme inhibition discontinues. In this study, the GSI MK-0752 was administered to conscious CMP rhesus monkeys in conjunction with in vivo stable-isotope-labeling, and dose-dependently reduced newly generated CNS Aβ. In contrast to systemic Aβ metabolism, CNS Aβ production was not increased after the GSI was cleared. These results indicate that most of the CNS Aβ was metabolized to products other than Aβ, including C-terminal truncated forms of Aβ: 1-14, 1-15 and 1-16; this demonstrates an alternative degradation pathway for CNS amyloid precursor protein during γ-secretase inhibition.

Introduction

The amyloid hypothesis proposes that the accumulation of amyloid-β (Aβ) in the brain leads to a cascade of events culminating in Alzheimer’s disease (AD) (Hardy and Selkoe, 2002; Golde, 2005). Aβ is generated by the sequential cleavage of amyloid precursor protein (APP) by β-secretase and γ-secretase (Blennow et al., 2006; Steiner et al., 2008; Wolfe, 2008b). Aβ accumulation in the form of insoluble plaques and soluble monomers and oligomers develops due to an imbalance in the production and clearance of these Aβ forms in the CNS.

Because Aβ is a major therapeutic target, multiple Aβ-lowering strategies are under development, such as increasing clearance (including Aβ immunization approaches) or decreasing production by inhibition of β-secretase or γ-secretase (Hock et al., 2003; Lemere et al., 2004; Vassar, 2004; Wolfe, 2008a; Tomita, 2009). γ-Secretase is a complex of integral membrane proteins that are present in most cells, but may have different functions depending on cell type (Steiner et al., 2008). γ-Secretase inhibitors (GSIs) target the γ-secretase complex to inhibit the production of Aβ from the β-C-terminal fragment of APP. However, GSIs also inhibit notch processing, thereby interfering with the critical role of notch in signaling cell differentiation in the immune system and gastrointestinal tract (Maillard et al., 2003; Stanger et al., 2005). Chronic inhibition with a GSI leads to mechanism-based toxic side-effects (Searfoss et al., 2003; Wong et al., 2004), which may be avoided by using partial or intermittent γ-secretase inhibition. However, there is a concern that when γ-secretase inhibition is partial or intermittent, accumulated APP products, including the β-C-terminal fragment, will result in Aβ overshoot or rebound.
After GSI treatment, a rebound in levels of Aβ above placebo is consistently observed in plasma of humans and animal models (Lanz et al., 2004, 2006; Michener et al., 2006; Rosen et al., 2006; Siemens et al., 2006). In addition, an early elevation in plasma Aβ40 has also been observed in rhesus monkeys at GSI doses that do not reduce plasma Aβ40 (Michener et al., 2006). Although an overshoot was reported in the CNS of a rat model (Burton et al., 2008), it has not been observed in the CNS of human volunteers (Rosen et al., 2006) or other animal models (Lanz et al., 2004). The contrasting results observed in plasma versus the CNS highlight the separation of the periphery from the CNS and indicate potentially different molecular mechanisms of Aβ production and APP processing. As increased concentrations of Aβ in the CNS are expected to be harmful, it is important to demonstrate that candidate compounds are truly targeting Aβ generation in the CNS. Clarification of the specific effect on the processing of APP metabolites is also important for the selection of optimal compounds for development. As few treatments or conditional experiments can be evaluated in humans, we translated a recently described stable-isotope-labeling kinetic (SILK) method (Bateman et al., 2006) to a nonhuman primate model for testing of therapeutics and molecular mechanisms. By administering stable-isotopes in conjunction with a GSI, the effect of inhibition on Aβ production was measured by liquid chromatography tandem mass spectrometry (LC-MS/MS). Similar to results seen in humans (Bateman et al., 2009), there was a dose-related decrease in Aβ production in response to γ-secretase inhibition without an overshoot of either newly generated or total levels of Aβ.

Materials and Methods

Cisterna magna ported rhesus monkey model. All procedures related to the use of animals in these studies were reviewed and approved by the Institutional Animal Care and Use Committee at Merck Research Laboratories at West Point, and conform with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, 1996). The chronically implanted cisterna magna catheter and port system provides for noninvasive collection of uncontaminated CSF samples and the capability for repeat sampling. This chronic system allows for longitudinal studies and comparison to historical data within the same monkeys. A customized flexible silicone catheter (SoloCath) is freely suspended in the cisterna magna, anchored firmly on both sides of the atlanto-occipital membrane, and tunneled subcutaneously to the midscapular region where it is fed into a surgically implanted port body. CSF is accessed by aseptically inserting a needle through the skin and membrane covering the port into the reservoir of the port body (Gilberto et al., 2003); CSF flows through the cannulation system without the need for active withdrawal. These monkeys are also implanted with a standard catheter system in the jugular vein (titanium vascular access port by SoloPort) for chronic vascular access used for the 13C6-leucine infusions.

13C6-Leucine infusion protocol. In preparation for each experiment, rhesus monkeys were restricted to a diet of vegetables and fruit for a 24 h period before the start of the 13C6-leucine infusion; all food was removed ~17 h before dosing. Vegetables and fruit were provided again after the 8 h sample collection (during the infusion) and the normal diet of monkey chow (includes protein) was given after the termination of the infusion (~12 h in the final standard protocol). Conscious monkeys were transferred to the laboratory and placed in primate restraint chairs; a 22 g Huber needle (Intech) connected to a sterile adapter was inserted into the port body to prepare the cisterna magna catheter and port system for CSF sampling. A sterile stock solution of 13C6-leucine (Cambridge Isotope Laboratories, CLM-2262) was made fresh daily for each study at 15 or 7.5 mg/ml in saline for the high (8 mg/kg + 8 mg/kg/h) and low (4 mg/kg + 4 mg/kg/h) leucine infusions, respectively. The 13C6-leucine was infused in the chronic vascular access port at ~0.5 ml/min or ~0.1 ml/min for the bolus and 12 or 21 h infusions (exact rate was normalized for body weight). Baseline CSF and blood samples were collected 1 h before the start of the 10 min primed bolus injection. In the final, optimized (12 h) protocol, plasma and CSF samples were also collected following the initiation of the 13C6-leucine infusion at 2 h (11:00 A.M.), 4 h (1:00 P.M.), 6 h (3:00 P.M.), 8 h (5:00 P.M.), 12 h (9:00 P.M., end of infusion), 15 h (12:00 A.M.), 18 h (3:00 A.M.), 21 h (6:00 A.M.), 24 h (9:00 A.M., day 2), 27 h (12:00 P.M.), 30 h (3:00 P.M.), 33 h (6:00 P.M.), 36 h (9:00 P.M., day 3) and 39 h (12:00 P.M.). CSF (1 ml) was collected per time point, and samples were separated into aliquots and transferred to various laboratories for blinded analyses.

γ-Secretase inhibitor study protocol. The Merck γ-secretase inhibitor, MK-0752, was previously characterized for in vivo pharmacokinetics and efficacy, and demonstrated decline of CSF Aβ40 levels in healthy human volunteers (Rosen et al., 2006). MK-0752 is a moderately potent γ-secretase inhibitor, showing dose-dependent reduction of Aβ40 with an IC50 of 5 nM in human SH-SY5Y cells. Dose-dependent reductions in plasma, brain and CSF Aβ40 were observed in guinea-pigs following single oral doses of 10–30 mg/kg (brain IC50 = 440 nM). Concentration required for 50% reduction of brain Aβ40 was 318 and 154 nM in APP-YAC and Tg2576 mice, respectively. For the purposes of the current study, 60 and 240 mg/kg dose of MK-0752 were selected to elicit intermediate and near complete CSF Aβ40 reduction for evaluating the potential effects on Aβ production and clearance. A 3-way crossover randomized design with 2 week intervals between doses in cisterna magna ported (CMP) rhesus monkeys was used (n = 6 male rhesus monkeys; n = 5 at 6–9 years and n = 1 at 17 years at time of study; 7–9.5 kg). MK-0752 (60 or 240 mg/kg) or vehicle (water) was administered orally to conscious, chaire rhesus monkeys 1 h before the initiation of the primed 12 h 13C6-leucine constant infusion (4 mg/kg + 4 mg/kg/h). CSF and plasma samples were collected as described above for determination of MK-0752 concentrations, Aβ40 (ELISA), 13C6-leucine labeling of Aβ, and free 13C6-leucine enrichment. To fully characterize the rebound beyond baseline in plasma Aβ40, a follow-up study was conducted. Six rhesus monkeys (n = 3/group) were treated orally with vehicle or 240 mg/kg MK-0752. CSF and plasma samples were collected as described for the optimized protocol with the addition of time points through 240 h postdose (48, 72, 144, 168, 216, 240 h).

Amyloid β ELISA. Solid black 96 well Nunc plates were coated with 3 µg/ml N-terminal Aβ antibody 6E10 (Signet) in pH 9.4 bicarbonate buffer overnight at 4°C, washed with PBS with 0.1% Tween 20, and blocked with 3% BSA in PBS (BSA/PBS) for 3 h. CSF samples for the Aβ1–40 assay were diluted 1:8 in BSA/PBS; 50 µl of samples and standards, starting at 4:00 P.M., were loaded onto the plate. CSF samples for Aβ1–42 were diluted 1:4 in BSA/PBS and 100 µl of samples and standards, starting at 2:00 P.M., were loaded onto the plate. Plasma samples were diluted 1:4 in BSA/PBS and 50 µl of samples and standards, starting at 4:00 P.M., were loaded onto the plate. Fifty microliters of G2-10-alkaline phosphatase (Aβ1–40) or 12F4-alkaline phosphatase (Aβ1–42) diluted in BSA/PBS was added to the plate and incubated overnight at 4°C. Plates were washed 3 times in PBS with 0.1% Tween 20 and 100 µl of alkaline phosphatase substrate Tropix CDP-star (Applied Biosystems) was added for 30 min. Luminescence was measured using a PerkinElmer Envision plate reader.

Amyloid β immunoprecipitation. Antibody beads were prepared by covalently binding 4G8 antibody (GE Healthcare) (recognizes Aβ residues 18-22) or 6E10 antibody (Covance) (recognizes Aβ residues 1-17) to CNBr Sepharose beads (GE Healthcare) at a concentration of 10 mg/ml antibody (according to the manufacturer’s protocol). The antibody beads were stored at 4°C in a 30% slurry of PBS 0.02% azide. 4G8 immunoprecipitation. CSF (500 µl) was diluted 1:1 with PBS. Protease inhibitors (40 µg/ml aprotinin and 20 µg/ml leupeptin) (Calbiochem), 30 µl of antibody-bead slurry, and guanidine (final concentration of 0.5 M) in Tris-HCl buffer pH 8 were added to the diluted CSF, and samples were rotated overnight at 4°C. The beads were rinsed once with 0.5× guanidine and twice with 25× ammonium bicarbonate, with centrifugation in between rinses. They were aspirated dry after the final rinse and Aβ was eluted from the antibody–bead complex using 50 µl of neat formic acid. After centrifuging the beads again, the formic acid
supernatant was transferred to a new tube and evaporated in a rotary evaporator at 37°C for 30 min. The dried extract was reconstituted in 20 μl of 9:1 ammonium bicarbonate (25 mM): acetonitrile. The extract was digested with 400 ng of sequence grade trypsin (Promega) prepared in 25 mM ammonium bicarbonate and incubated at 37°C for 18 h.

6E10 immunoprecipitation. Protease inhibitors and guanidine were added to CSF (900 μl) as described above. In addition, samples were spiked with 100 fmol (10 fmol/μl) of isotope-labeled Aβ peptide Aβ1-15[Ala(U-13C3,15N), Arg(U-13C6, U-15N4),Gly(U-13C2, 15N)] (prepared in HPLC grade water) (Anaspec) before addition of 30 μl of 6E10 bead slurry, after which they were rotated overnight at 4°C. The beads were rinsed as described above and peptides and proteins were eluted from the antibody–bead complex using 50 μl of neat formic acid. After centrifugation, the formic acid supernatant was transferred to autosampler vials and evaporated in a rotary evaporator at 37°C for 30 min. The dried extract was reconstituted in 20 μl of 5% acetonitrile in 0.1% formic acid.

Liquid chromatography-mass spectrometry. Aβ extracts were analyzed on a Thermo-Finnigan LTQ equipped with a New Objective nanoflow ESI source. Peptides were separated by RP HPLC using an Eksigent 2D-LC nanoflow pump operating in 1D mode at a flow of 200 nl/min. Five microliters of extract was injected onto a New Objective picofrit column packed to 10 cm with 5 μm Magic C18aq packing material (Michrom). Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B consisted of 0.1% formic acid in acetonitrile. The doubly charged species of Aβ 1-14, 1-15, 1-16, and 1-17 were separated by applying a linear gradient from 2 to 2% B by 40 min.

Calculation of labeled protein ratio and quantitation of Aβ1-14, 1-15, 1-16, and 1-17. Percentage of labeled Aβ was calculated as the ratio of all b- and y-tandem MS ion intensities from 13C6-Aβ 17–28 (m/z 666.5) divided by all b- and y-tandem MS ion intensities from Aβ 17–28 (m/z 663.5) as previously described (Bateman et al., 2007b). The peak areas for Aβ1-14, 1-15, 1-16, and 1-17 (MS² total ion currents) were calculated and normalized to the Aβ1-15 isotope-labeled standard using Xcalibur’s quantitation software. Results were exported to MS Excel. All statistical calculations were performed with GraphPad 5.0. The coefficient of variation for quantitation was ±3.8% for triplicate injections of the same sample.

Gas chromatography-mass spectrometry and calculation of 13C6-leucine enrichment. Plasma and CSF 13C6-leucine enrichment was quantified using capillary gas chromatography mass spectrometry (GC-MS; Agilent 6890N gas chromatograph and Agilent 5973N mass selective detector). Leucine was converted to the heptfluorobutyric propyl ester derivative; 13C6-leucine (m/z 349 and 355) enrichment was quantified using GC-MS in negative chemical ionization mode (Yarasheski et al., 1992; Bateman et al., 2007b). 13C6-Leucine enrichment was calculated as the tracer/tracer ratio (Wolfe et al., 2005). The GC-MS instrument response was calibrated using gravimetric standards of known isotope enrichment.

Calculations of fractional synthesis rate, fractional clearance rate, and newly generated Aβ. The fractional synthesis rate (FSR) and fractional clearance rate (FCR) were calculated in the traditional manner (Wolfe et al., 2005). FSR equals the slope of the labeled Aβ (hours 2–8) divided by plasma 13C6-leucine enrichment (precursor pool for Aβ synthesis) during leucine infusion (hours 2–8). FCR equals the slope of the natural logarithm of labeled Aβ during the clearance phase (hours 18–33). Newly generated Aβ was calculated as previously described as the product of the percentage of labeled Aβ (LC-MS results) and the total Aβ concentration (ELISA results) (Bateman et al., 2009). All statistical calculations were performed with GraphPad 5.0.

Results
Translation of human CNS-SILK method for use in rhesus monkeys
We adapted the human in vivo SILK method for use with CMP rhesus monkeys. A summary of the protocol is shown in Figure 1. 13C6-Leucine is intravenously administered to CMP rhesus monkeys for 12 h; CSF is collected from the cisterna magna port during and after administration (Fig. 1A, B). Labeled leucine enrichment in plasma and CSF is measured by GC-MS (Yarasheski et al., 1992) (Fig. 1C). The incorporation of the label into Aβ is quantitated by immunoprecipitation paired with LC-MS (Bateman et al., 2006, 2007b) (Fig. 1D), and the results are used to calculate production and clearance rates.

To translate the in vivo method from humans to monkeys, and to achieve similar 13C6-Aβ labeling, two 13C6-leucine infusion protocols were evaluated. CMP rhesus monkeys were infused with 13C6-leucine (8 mg/kg/h) for either 21 or 12 h, and CSF was collected approximately every 3 h for up to 48 h (Fig. 2). The administration of 8 mg/kg/h 13C6-leucine resulted in fourfold higher plasma 13C6-leucine labeling than the ratios observed in humans (Bateman et al., 2006). Reduction of the infusion to 4 mg/kg/h 13C6-leucine for 12 h resulted in maximum plasma 13C6-leucine labeling of 15%, which is more similar to human studies, and thus was used for subsequent studies. The steady-state labeling of Aβ exceeded the theoretical maximum when CSF 13C6-leucine levels were used as the precursor pool (Fig. 2A, 21 h infusion). In contrast, the steady-state labeling of Aβ approached and did not exceed the maximum when using plasma 13C6-leucine levels (Fig. 2B, 21 h infusion). For this reason, subsequent studies used plasma 13C6-leucine levels for labeled Aβ normalization.
tion. By reducing the $^{13}$C$_6$-leucine infusion from 21 to 12 h, more sample time points could be added to the Aβ clearance phase which enabled more accurate clearance rate calculations.

Intrasubject and intersubject variability for measurements of Aβ metabolism were analyzed and calculated FCRs were compared with steady-state levels as measured by ELISA. The same three animals were evaluated in two separate studies, and calculated FCRs were correlated between studies ($r = 0.9969$; supplemental Fig. 1A, available at www.jneurosci.org as supplemental material). Different individual monkeys (n = 3) also showed similar FCRs with SEM between 0.3 and 0.5%/h demonstrating some biological variability between monkeys (supplemental Fig. 1B, available at www.jneurosci.org as supplemental material). FSR and FCR results were compared with levels of Aβ. There was a positive correlation between FSR and FCR values and levels of Aβ ($n = 9$, FSR vs Aβ 40 Pearson $r = 0.76$, $p = 0.016$; FSR vs Aβ42 Pearson $r = 0.77$, $p = 0.015$; FCR vs Aβ 40 Pearson $r = 0.41$, $p = 0.27$; FCR vs Aβ 42 Pearson $r = 0.79$, $p = 0.03$).

The 12 h optimized $^{13}$C$_6$-leucine (4 mg/kg/h) infusion (n = 12 monkeys) demonstrated low intersubject variability for Aβ labeling kinetics at each time point sampled over 39 h (average SEM = 0.02, n = 12) (Fig. 3A). There were no significant differences observed between genders or across ages of the animals included in this study. The mean FSR (h 2–8) was 10.7 ± 0.6%/h (SEM) and the mean FCR (h 18–33) was 9.9 ± 0.5%/h (Fig. 3B); these values were not significantly different ($p = 0.25$).

**CNS-penetrant γ-secretase inhibitor decreases the generation of newly produced Aβ in the brain of rhesus monkeys**

In a three-way crossover study, healthy male CMP rhesus monkeys (n = 6) were treated with single oral administration of two doses of the CNS-penetrant GSI, MK-0752, and vehicle to evaluate effects on Aβ metabolism. MK-0752 concentrations were quantified in plasma (Fig. 4A) and CSF (Fig. 5A), showing peak concentration at 2 h with both doses. The majority of the drug was cleared from plasma and CSF by 36 h. Effects of this GSI on Aβ concentrations in the plasma and CSF were quantified using ELISA and demonstrate a dose-related reduction of Aβ levels in plasma (Aβ40) ($p = 0.0012$) and CSF (Aβ40 and Aβ42) ($p < 0.001$) as determined by a repeated-measures ANOVA (Figs. 4B, 5B, C). Plasma Aβ levels rebounded above baseline after GSI inhibition (60 mg/kg) ($p < 0.001$ paired t test vehicle vs 60 mg/kg h 33–48) (Fig. 4B); in contrast, CSF Aβ levels did not overshoot baseline levels (Figs. 5B,C). In an extended study designed to evaluate the rebound response of the higher dose of 240 mg/kg, an overshoot above baseline was observed in plasma 48 h after treatment ($p < 0.001$ unpaired t test vehicle vs 240 mg/kg h 48–240) (Fig. 4D).

The GSI dose-dependently reduced newly synthesized CNS Aβ over 48 h, without any evidence of a rebound or overshoot of newly generated CNS Aβ (Fig. 6A). Similar results were observed in an extended study (240 h) with the 240 mg/kg GSI treatment (Fig. 6B) (unpaired t test, $p = 0.0009$). Area under the curve (AUC) analysis for newly generated Aβ indicated a significant difference between the means of the vehicle and GSI treatment groups (ANOVA, $p < 0.0001$). The mean AUC not only reflected a dose-dependent decrease during the $^{13}$C$_6$-leucine infusion (0–12 h) (Fig. 6C), but also during the entire study (0–48 h) (Fig. 6D) (Tukey post hoc analysis, $p < 0.001$). During the 48 h sampling period, the mean AUC of newly generated Aβ was reduced by 49% in the 60 mg/kg dose group and by 90% in the 240 mg/kg GSI dose group ($p < 0.01$) (Fig. 6D).

**CNS-penetrant γ-secretase inhibitor alters levels of noncanonical APP fragments**

To evaluate alternative pathways of APP degradation in the presence of γ-secretase inhibition, noncanonical forms of Aβ (e.g., Aβ not produced by β- and γ-secretase cleavage) were measured in the CSF of rhesus monkeys (n = 6, 3/group) treated with a high dose of MK-0752 (240 mg/kg) versus vehicle. Four isoforms of Aβ (Aβ 1-14, Aβ 1-15, Aβ 1-16, and Aβ 1-17) were quantified from the CSF using internal standard controls. In animals treated with the GSI, levels of Aβ 1-14, Aβ 1-15, and Aβ 1-16 increased over vehicle levels, while levels of Aβ 1-17 decreased (unpaired t test, $p < 0.0001$, Fig. 7). The expected reductions in plasma Aβ40 and CSF Aβ40 and Aβ42 were demonstrated during the same time (Figs. 4D, 5E,F), while plasma Aβ40 rebounded after 48 h (Fig. 4D).
generation of CNS Aβ was significantly reduced in response to γ-secretase inhibition. However, in contrast to the periphery, production of CNS Aβ did not rebound above baseline after cessation of inhibition. Defining the metabolic fate of APP in the CNS is critically important for the development of γ-secretase inhibitors to treat AD, as a substrate build-up of APP fragments could potentially lead to an overshoot in neurotoxic amyloid peptides. The lack of Aβ rebound in the CNS could be attributed to the shunting of APP (possibly β C-terminal fragments, e.g., C99) to γ-secretase independent degradation. In support of this alternative, noncanonical processing, increased levels of APP fragments Aβ1-14, 1-15, and 1-16 were observed in CSF samples of animals treated with the GSI, while Aβ1-17 was decreased. The CNS elimination of APP through nonamyloidogenic pathways strengthens the case for the use of γ-secretase as a therapeutic target for AD and may also be relevant for physiological processing of APP.

Many treatments have demonstrated efficacy in preclinical AD murine models; however, to date, none have been successful in clinical trials (Duff and Suleman, 2004). Failed translation from preclinical models to clinical studies may be a result of several reasons, including targeting of the wrong molecular mechanism of disease, failure to sufficiently modulate the target, or administration of the therapy too late in the course of the disease (Bateman and Klunk, 2008). For Alzheimer’s disease, one limiting factor for translation from preclinical models to clinical trials is the lack of animal models that closely reflect human Aβ CNS physiology; such models would provide relevant information regarding the target, mechanism of action, and therapeutic effectiveness. Thus, there is a need to develop better models of therapeutic targets that would provide a bridge for the translation of murine models of amyloidosis to human trials in AD.

The CMP rhesus monkey is a nonhuman primate model that enables repeat sampling of CSF (Gilberto et al., 2003; Nantermet et al., 2009; Sankaranarayanan et al., 2009). Pairing this model with in vivo SILK enables direct measurement of newly generated CNS proteins and peptides such as Aβ. Results demonstrate that Aβ metabolism in the rhesus monkey is very similar to humans (Bateman et al., 2006), which is expected because there are no significant amyloid plaques present in the rhesus monkey brain at this age (Struble et al., 1985). In conjunction with in vivo stable-isotope-labeling, new
This combination allows for the use of fewer monkeys to clearly answer research questions (Fig. 3; supplemental Fig. 1, available at www.jneurosci.org as supplemental material).

There are similarities and differences between the metabolism of Aβ in rhesus monkeys and humans. As expected, the average FSR and FCR are balanced in both the rhesus monkey (n = 12) (Fig. 3B) and human (Bateman et al., 2006). However, rhesus monkey CNS FSR and FCR are slightly faster than human CNS FSR and FCR (~10% per hour vs ~8% per hour, respectively; Fig. 3B) (Bateman et al., 2006). Other than species differences, the observed disparity in Aβ metabolism may be due to CSF sampling location. Human CSF was sampled by lumbar intrathecal catheter, whereas monkey CSF was sampled at the cisterna magna at the base of the head (Fig. 1A). The more proximal sampling location could also explain the slightly shorter delay in 13C6 incorporation observed in the monkeys versus that seen in humans (4 h vs 5 h, respectively), which indicates that lumbar sampling only slightly delays the appearance of newly generated Aβ. Prior human studies demonstrated individual changes in CSF Aβ levels by 100–400% over several hours (Bateman et al., 2007a). In this study of nonhuman primate CSF, there were individual CSF Aβ changes of 30–50%. The group changes in Aβ levels were similar between human lumbar CSF samples and nonhuman primate CMP samples when averaged (25–50% over a 24 h period). However, CMP monkey samples did not demonstrate a progressive rise in Aβ levels. Possible reasons for differences in the intrasubject CSF Aβ variability and lack of CSF Aβ rise include species differences, behavioral activity, site of sampling, frequency or amount of sampling, and sleep/wake cycle differences.

Findings in the nonhuman primate model also have implications for human studies. For example, in this study, we determined that plasma is more accurate than CSF for leucine precursor measurements of CNS Aβ generation (Fig. 2). As the CSF normalized ratio exceeded the theoretical maximum of one, the CSF 13C6-leucine is an underestimate of the precursor pool labeling. The labeled leucine precursor enrichment in monkey plasma is higher than CSF, similar to results observed in humans (Bateman et al., 2006). This suggests that plasma-labeled leucine measurements are more accurate for calculations of Aβ metabolism in human, as well as the nonhuman primate.
The utility of this CMP rhesus monkey translational model was demonstrated by quantifying Aβ metabolism before and after acute exposure to the GSI MK-0752. A prior human GSI SILK study indicates that a decrease in Aβ production can be measured to a sensitive degree (Bateman et al., 2009). In this nonhuman primate study, GSI efficacy (blocking Aβ production) and duration was higher compared with that observed in the human GSI study. Possible reasons for these observed differences include the GSI compound used, the dose of GSI administered, and, possibly, species differences. The extended effect may be due to the longer half-life of MK-0752 (10 h) in rhesus monkey versus LY450139 (2.5 h) in human (Siemers et al., 2005).

Consistent with previous observations (Lanz et al., 2004, 2006; Michener et al., 2006; Rosen et al., 2006; Siemers et al., 2006; Burton et al., 2008), plasma Aβ levels did rebound after the GSI was cleared (Fig. 4). However, concentrations of Aβ in the CSF did not overshoot baseline or placebo Aβ levels (Fig. 5). Measurement of production rates directly confirms that this GSI reduced Aβ levels by decreasing the generation of new Aβ without a subsequent rebound (Fig. 6). These results suggest that the Aβ precursor is being degraded by another pathway during inhibition (Portelius et al., 2009). In vitro studies have suggested that in the presence of γ-secretase inhibition, β-secretase and α-secretase generate Aβ 1-14, 1-15, and 1-16, and that Aβ isoforms longer than 1-16 are reduced (Portelius et al., 2009). Our results support this hypothesis by demonstrating for the first time in a nonhuman primate model, the rise in Aβ 1-14, 1-15, and 1-16 with a concurrent decrease of Aβ 1-17 (Fig. 7). Further, the course of altered APP metabolites indicates that Aβ 1-17 is possibly a cleavage product of Aβ 1-40 and Aβ 1-42, as all are decreased at similar times (0–48 h), while Aβ 1-14, 1-15, and 1-16 are increased during and after inhibition (0–144 h). Potential candidates known to cleave at the Aβ 1-17 site include endothelin converting enzyme, insulin-degrading enzyme, and matrix metalloproteinase-9 (Yan et al., 2006). These findings advance our understanding of the mechanisms of APP processing in the CNS, and provide novel information about the effects of GSI therapy on APP processing.

In conclusion, our study demonstrates that the effect of therapies developed to target Aβ production or clearance can be evaluated directly in a preclinical, nonhuman primate model. This will aid in the selection of clinical candidate compounds, and optimization of dose and timing of drug administration in a model which is similar to human Aβ physiology. In addition, molecular mechanisms of CNS protein processing can be explored by using therapeutics, which modulate key CNS enzymes with physiologically relevant models. These methods and findings hold promise to improve the likelihood of successful clinical trials for the treatment of Alzheimer’s disease and other CNS disorders.

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