Brain and Circulating Levels of Aβ1–40 Differentially Contribute to Vasomotor Dysfunction in the Mouse Brain

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Background and Purpose—Amyloid-β (Aβ), a peptide that accumulates in the brain and circulates in the blood of patients with Alzheimer disease, alters the regulation of cerebral blood flow and may contribute to the brain dysfunction underlying the dementia. However, the contributions of brain and circulating Aβ1–40 to the vascular dysfunction have not been elucidated.

Methods—We used transgenic mice overexpressing mutated forms of the amyloid precursor protein in which Aβ1–40 is elevated in blood and brain (Tg-2576) or only in brain (Tg-SwDI). Mice were equipped with a cranial window, and the increase in cerebral blood flow induced by neural activity (whisker stimulation), or by topical application of endothelium-dependent vasodilators, was assessed by laser-Doppler flowmetry.

Results—The cerebrovascular dysfunction was observed also in Tg-SwDI mice, but despite ≈40% higher levels of brain Aβ1–40, the effect was less marked than in Tg-2576 mice. Intravascular administration of Aβ1–40 elevated plasma Aβ1–40 and enhanced the dysfunction in Tg-SwDI mice, but not in Tg-2576 mice.

Conclusions—The results provide evidence that Aβ1–40 acts on distinct luminal and abluminal vascular targets, the deleterious cerebrovascular effects of which are additive. Furthermore, the findings highlight the importance of circulating Aβ1–40 in the cerebrovascular dysfunction and may provide insight into the cerebrovascular alterations in conditions in which elevations in plasma Aβ1–40 occur. (Stroke. 2013;44:198-204.)

Key Words: β-Amyloid ■ cerebral blood flow ■ somatosensory cortex ■ Tg-2576 ■ Tg-SwDI

There is increasing evidence that the regulation of the cerebral circulation is disrupted in Alzheimer disease (AD). Although resting cerebral blood flow (CBF) is reduced in selected brain regions of AD patients, the increases in CBF induced by neural activity are attenuated early in the course of the disease. Studies in mice overexpressing mutated forms of the amyloid precursor protein (APP) have indicated that the Amyloid-β (Aβ) peptide, Aβ1–40 in particular, alters key factors regulating CBF. Thus, the increases in CBF induced by neural activity or by endothelium-dependent vasodilators are attenuated in these mice. Furthermore, the ability to keep CBF independent of changes in arterial pressure (cerebrovascular autoregulation) is profoundly disrupted. These findings have raised the possibility that Aβ leads to brain dysfunction, not only by its deleterious effects on neurons and glia, but also by reducing cerebrovascular reserves and increasing the susceptibility of the brain to injury.

In AD patients, Aβ is elevated both in brain and plasma. Furthermore, plasma Aβ is also elevated in cerebral amyloid angiopathy, small vessel disease, and Down syndrome. However, the relative contribution of plasma and brain Aβ to the cerebrovascular dysfunction has not been defined. In particular, it is unclear whether increases in brain Aβ1–40 are necessary and sufficient to alter cerebrovascular regulation or whether elevations both in brain and circulating Aβ are needed. Therefore, it would be of interest to determine the cerebrovascular effects of elevations in plasma Aβ in the context of elevated brain Aβ.

Mice expressing the Swedish, Dutch, and Iowa APP mutations under the control of the Thy1.2 neuronal promoter (Tg-SwDI) have Aβ increases in brain but not in plasma. In contrast, Tg-2576 mice, which express APP with the Swedish mutation driven by a prion protein promoter, have elevations in brain and plasma Aβ. We used Tg-SwDI and Tg-2576 mice to investigate the relative contribution of brain and circulating Aβ1–40 to the cerebrovascular dysfunction. We found that elevations in brain Aβ1–40 are sufficient to alter cerebrovascular regulations. However, circulating Aβ1–40 enhances the cerebrovascular dysfunction induced by brain Aβ1–40. The findings provide the first evidence for distinct luminal and abluminal targets mediating the deleterious cerebrovascular effects of circulating and brain Aβ, and provide...
insight into the cerebrovascular alterations in conditions associated with chronic elevations of circulating Aβ.

Materials and Methods

Mice
All procedures were approved by the Institutional Animal Care and Use Committee of Weill Cornell Medical College. Studies were performed in 3 to 4 month-old Tg-2576,17 Tg-SwDI transgenics,16 and their littersmates. Because Tg-2576 mice are on a congenic 129/S6 background and Tg-SwDI mice are on a congenic C57BL6 background,16,17 we first tested cerebrovascular responses in transgene-negative age-matched wild-type (WT) littersmates of both transgenic backgrounds. No differences between transgene-negative 129/S6 and C57BL/6J in CBF responses were observed and the results from the WT mice were pooled.

General Surgical Procedures
As described in detail elsewhere,16,17 mice were anesthetized with isoflurane (1%–2%, vol/vol). A femoral artery was cannulated for recording of arterial pressure and collection of blood samples. In some studies, the external carotid artery ipsilateral to the cranial window was catheterized for intracarotid infusion of human Aβ1–40 (see below).18 Mice were intubated and artificially ventilated with an O2/CO2 mixture adjusted to provide an arterial PCO2 of 100 to 120 mm Hg. A femoral vein was cannulated for intravenous injections of test agents. A carotid artery was cannulated for arterial pressure monitoring using a thermostatically controlled rectal probe connected to a heating pad. After surgery, isoflurane was discontinued and anesthesia was maintained with urethane (750 mg/kg; IP) and α-chloralose (50 mg/kg; IP). The level of anesthesia was monitored by testing corneal reflexes and motor responses to tail pinch.

Monitoring of Cerebral Blood Flow
The somatosensory cortex was exposed by drilling a small opening through the parietal bone (2×2 mm), the dura was removed, and the site was superfused with modified Ringer solution (37°C; pH 7.3–7.4).5,21,22 CBF was continuously monitored at the site of superfusion through the parietal bone (2×2 mm), the dura was removed, and the site was superfused with modified Ringer solution (37°C; pH 7.3–7.4).5,21,22 CBF was continuously monitored at the site of superfusion with a laser-Doppler flow probe (Vasamedic) positioned stereotaxically above the cortical surface and connected to a computerized data acquisition system. CBF values were expressed as percent increase relative to baseline.5,22

Measurement of Brain and Plasma Aβ
Brain and plasma Aβ levels were determined using ELISA-based assays, as described previously.16,19 Briefly, cerebral hemispheres were sonicated and centrifuged, and Aβ1–40 concentration (pmol/mg) was determined using the 2G3/3D6 and m21F12/3D6 sandwich ELISA assay (antibody reagents were generously provided by Lilly Research Laboratories). For determination of plasma concentrations, plasma samples were treated with 0.5x vol/vol of 5 mol/L guanidine HCl for 30 minutes at room temperature, and Aβ1–40 concentration (pmol/mL) was determined as described above for brain Aβ1–40.

Immunohistochemistry
Anesthetized mice were perfused transcardially with heparinized saline, followed by 4% (wt/vol) paraformaldehyde.18,20 Brains were removed, postfixed, and sectioned (thickness of 40 µm). Free-floating sections were randomly selected and processed for labeling endothelial cells with glucose transporter-1 (rabbit antiglucose transporter-1,1:500; EMD Chemicals). The specificity of the labeling was established by omitting the primary antibody or by preabsorption with the antigen. Images were acquired using a confocal laser scanning microscope (Leica) in somatosensory cortex underlying the cranial window (0.38 to −1.94 mm from Bregma). Brain sections from Tg-2576, Tg-SwDI, and WT littersmates were processed under identical conditions and imaged using identical settings. The number of glucose transporter-1-positive vascular profiles and the % area occupied by the profiles were quantified using ImageJ (National Institutes of Health).

Experimental Protocol for CBF Experiments
CBF recordings were started after arterial pressure and blood gases were in a steady state (Supplemental Table I). All pharmacological agents studied were dissolved in Ringer solution, unless otherwise indicated. To study the increase in CBF produced by somatosensory activation, the whiskers were activated by side-to-side deflection for 60 seconds. The endothelium-dependent vasodilators acetylcholine (10 µmol/L; Sigma), A23187 (3 µmol/L), and bradykinin (50 µmol/L) were topically superfused for 3 to 5 minutes and the resulting changes in CBF monitored.5,22 CBF responses to the smooth muscle relaxant adenosine (400 µmol/L; Sigma) were also examined.16,17 In experiments with intracarotid infusion of human Aβ1–40 (rPeptides; in dimethyl sulfoxide, final dimethyl sulfoxide concentration <0.05%), CBF responses were first tested without infusion. Then, vehicle or Aβ1–40 (1 µmol/L, 150 µL/h) was infused for 30 to 40 minutes into the internal carotid artery and responses were tested again (Figure 1).

Data Analysis
Data are expressed as mean±SEM. Two-group comparisons were analyzed by the 2-tailed t test. Multiple comparisons were evaluated by the ANOVA and Tukey test. Differences were considered statistically significant for probability values <0.05.

Results
Brain Aβ1–40 is Sufficient to Induce Cerebrovascular Dysfunction
In agreement with previous studies, Aβ1–40 levels were elevated in brain and plasma in 3 to 4 month-old Tg-2576 mice (Figure 2). However, in comparably aged Tg-SwDI mice Aβ1–40 levels were elevated only in brain, an increase more pronounced than in Tg-2576 (Figure 2B). The increases in CBF induced by whisker stimulation or endothelium-dependent vasodilators (acetylcholine, A23187, and bradykinin) were attenuated in Tg-SwDI mice (Figure 3A; Supplemental Figure I), but the attenuation was less pronounced than in Tg-2576 mice (Figure 3A; Supplemental Figure I). The CBF response to adenosine was not altered in either transgenics (Figure 3A), suggesting that the attenuation in vasomotor responses was not attributable to a nonspecific impairment of vascular smooth muscle reactivity or vascular damage. In support of this conclusion, no differences...
in the morphology and number of cerebral microvessels were observed in the somatosensory cortex of Tg-SwDI and Tg-2576 mice (Figure 3B and 3C).

Elevation in Plasma Aβ1–40 Induces Cerebrovascular Dysfunction in WT Mice

Next, we investigated the role of plasma Aβ1–40 in the cerebrovascular dysfunction. In WT mice, intracarotid infusion of human Aβ1–40 elevated plasma Aβ1–40 to levels comparable to those observed in Tg-2576, without increasing brain Aβ1–40 (Figure 2C and 2D). Aβ1–40 intracarotid infusion attenuated the increase in CBF induced by whisker stimulation and acetylcholine (Figure 4A) (P>0.05), but did not alter resting CBF or the CBF response to adenosine (Figure 4A; Supplemental Figure IIA). Therefore, circulating Aβ1–40 is sufficient to induce cerebrovascular dysfunction.

Elevation of Plasma Aβ1–40 Aggravates Cerebrovascular Dysfunction in Tg-SwDI, but Not in Tg-2576 Mice

To determine whether circulating Aβ1–40 and brain Aβ1–40 act synergistically on cerebrovascular function, we examined

**Figure 2.** Amyloid-β (Aβ)1–40 levels in plasma (A) and brain (B) of Tg-SwDI and Tg-2576 mice. Effect intracarotid infusion of Aβ1–40 on plasma (C) and brain (D) levels in wild-type (WT), Tg-SwDI and Tg-2576 mice (*P<0.05; from vehicle; ANOVA and Tukey test; n=5/group).

**Figure 3.** A, Increases in cerebral blood flow (CBF) elicited by whisker stimulation acetylcholine, (ACh; B), and Adenosine in wild-type (WT), Tg-SwDI, and Tg-2576 mice (*P<0.05 from WT; # P<0.05 from WT; and Tg-SwDI; ANOVA and Tukey test; n=5/group). B, Glucose transporter-1 (Glut-1) immunoreactivity in the somatosensory cortex of WT, Tg-SwDI, and Tg-2576 mice. C, Number of vascular profiles and % area occupied by blood vessels do not differ among the groups (P>0.05; n=4–5/group).
the cerebrovascular effects of elevation of plasma Aβ1–40 in Tg-SwDI mice. In Tg-SwDI mice, Aβ1–40 intracarotid infusion induced plasma Aβ1–40 elevations comparable to those of Tg-2576 mice (Figure 2C) and attenuated cerebrovascular responses to levels not different from Tg-2576 mice (Figure 4B). In contrast, in Tg-2576 mice Aβ1–40 infusion did not aggravate the cerebrovascular dysfunction, despite a substantial increase in plasma Aβ1–40 (Figures 2C and 4C). Infusion of Aβ1–40 did not affect brain levels of Aβ1–40 in Tg-SwDI or Tg-2576 mice (Figure 2D). Similarly, the Aβ infusion did not affect resting CBF or the CBF increase produced by adenosine (Figure 4B and 4C; Supplemental Figure II B and II C).

Discussion

Novel Findings of the Study

We found that elevations in brain Aβ1–40, as observed in Tg-SwDI mice, are sufficient to induce cerebrovascular alterations. However, the cerebrovascular dysfunction is less marked than that of Tg-2576 mice despite higher brain Aβ1–40 concentrations. Elevation in circulating Aβ1–40 in Tg-SwDI mice enhances the vasoactivity dysfunction to levels comparable to those of Tg-2576 mice. In contrast, further elevations in circulating Aβ1–40 in Tg-2576 mice do not aggravate the cerebrovascular dysfunction. These novel observations demonstrate that: (1) both brain and circulating Aβ1–40 are capable of inducing cerebrovascular dysfunction; (2) their effects are distinct and additive; (3) reach a maximum at the concentrations achieved in Tg-2576 mice.

Exclusion of Potential Sources of Artifacts

The findings of the present study cannot be attributed to differences in the physiological variables of the mice, because arterial pressure, blood gases, and body temperature were monitored and did not differ among the groups studied. Similarly, the differences in the cerebrovascular responses between Tg-2576 and Tg-SwDI mice are unlikely to be a consequence of differences in smooth muscle relaxation because the CBF response to the smooth muscle relaxant adenosine was preserved in both transgenics. Tg-SwDI mice exhibit amyloid deposition primarily in cerebral microvessels, whereas Tg-2576 mice develop amyloid deposition in pial and meningeal vessels. However, such differences in Aβ deposition are not relevant to the present study because mice were studied at 3 months of age, prior to development of amyloid angiopathy. Similarly, gross morphological
alterations of the cerebral microvasculature are unlikely to explain the observed differences in vascular reactivity because no differences were observed in the microvessels involved in the vascular responses studied.

**Contribution of Plasma and Brain Aβ1–40 to the Cerebrovascular Dysfunction**

We found that the alterations in functional hyperemia and endothelium-dependent responses in Tg-SwDI mice were less marked than in Tg-2576 mice, despite ≈40% higher brain Aβ levels. Considering that plasma Aβ1–40 is not measurable in Tg-SwDI but is elevated in Tg-2576, we hypothesized that the absence of circulating Aβ1–40 could explain the difference in the cerebrovascular dysfunction. Consistent with this prediction, intracarotid infusion of Aβ1–40 raised plasma Aβ1–40 up to the concentration observed in Tg-2576 mice and enhanced the dysfunction in Tg-SwDI mice to levels identical to those observed in Tg-2576 mice. Aβ1–40 can cross the blood-brain barrier in both directions and administration of exogenous Aβ1–40 into the circulation could conceivably enter the brain especially if the blood-brain barrier is altered. However, in our experiments the observed effects were not attributable to changes in brain levels because infusion of Aβ1–40 did not augment brain Aβ1–40 levels. Interestingly, infusion of Aβ1–40 in Tg-2576 mice increased plasma Aβ1–40 further, but failed to aggravate the cerebrovascular dysfunction. These observations, collectively, indicate that although brain or blood Aβ1–40 are sufficient to induce cerebrovascular dysfunction, their effects are additive and maximal at the concentrations reached in Tg-2576 mice.

**Cellular Mechanisms of the Cerebrovascular Effects of Brain and Plasma Aβ1–40**

The present findings provide evidence that circulating and brain Aβ1–40 act on distinct luminal and abluminal sites to induce cerebrovascular dysfunction. However, the cellular substrates underlying such effects on opposite sides of the vessel wall remain to be defined. Increasing evidence implicates oxidative stress mediated by CD36-induced activation of a Nox2-containing nicotinamide adenine dinucleotide phosphate oxidase. In Tg-2576, in which both brain and plasma levels of Aβ1–40 are elevated, deletion of CD36 or Nox2 rescues the cerebrovascular alterations completely, suggesting that reactive oxygen species are involved in the cerebrovascular effects of both blood and brain Aβ1–40. However, the cellular localization of CD36 and Nox2 has not been clarified in full. Studies with intracarotid administration of Aβ1–40 and in endothelial cell cultures suggest that the effects of circulating Aβ1–40 involve activation of CD36 and Nox2 in cerebral endothelial cells leading to vascular oxidative stress. However, it remains unclear how brain Aβ1–40 exerts its vascular action from the abluminal side of the vessel. One possibility is that parenchymal Aβ1–40, which is cleared through the perivascular space, acts on perivascular cells expressing CD36, that is, microglia and macrophages, which, in turn, contribute to vascular oxidative stress. In this case, brain and circulating Aβ1–40 would act on different targets on opposite sides of the vessels wall to induce cerebrovascular dysfunction. Another scenario is that circulating Aβ1–40 acts on circumventricular organs, which are devoid of blood-brain barrier, and exert their cerebrovascular effects through release of vasopressin from the paraventricular hypothalamus and cerebrovascular endothelin upregulation, as recently described for angiotensin-II. These possibilities need to be examined in future studies.

**Does Soluble Aβ Cause Cognitive Dysfunction?**

The deleterious cognitive effects of vascular and parenchymal amyloid deposition are well established, but the clinical correlates of soluble Aβ remain less clear. Amyloid deposits and their attendant vascular and parenchymal effects are detectable in patients by imaging, but is not yet possible to monitor soluble Aβ in its different states of aggregation. However, studies of human cerebral arteries have demonstrated that soluble Aβ induces alteration in vascular tone. Considering that in AD patients, as in APP mice, soluble Aβ is present in brain and cerebral blood vessels prior to amyloid deposition, it is conceivable that soluble Aβ has vascular effects also in humans. Indeed, soluble Aβ in low-order aggregation states (monomer, dimers, etc.) have emerged as key pathogenic factors in AD, and oligomeric Aβ is likely to alter both neuronal and vascular function.

**Potential Limitations of the Study**

One limitation of the present study is that the levels of exogenous Aβ1–40 in plasma producing cerebrovascular dysfunction and observed in Tg-2576 are higher than those observed in AD, small vessel disease, cerebral amyloid angiopathy, or Down syndrome. Therefore, it remains unclear whether the levels of Aβ present in AD patients would be sufficient to induce vascular dysfunction. However, cerebral blood vessels of AD patients are exposed to elevated plasma Aβ levels for years, and lower concentrations may be effective with a more prolonged exposure. Interestingly, AD immunotherapy can increase plasma Aβ levels up to 1000 folds, resulting in Aβ levels closer to those observed in Tg-2576 mice. Alterations in vascular structure and function are well known to occur in patients treated with Aβ antibodies, which has called for developing a better understanding of the intravascular effects of Aβ. The present findings demonstrate that circulating Aβ1–40 aggravates the cerebrovascular dysfunction induced by brain Aβ, potentially impeding the clearance of brain Aβ through the vascular pathway. Therefore, our data raise the possibility that increasing the clearance of plasma Aβ or counteracting its deleterious vascular actions could enhance the potential beneficial effects of Aβ immunotherapy. A caveat, however, is that after immunotherapy most plasma Aβ is antibody bound and it is unclear whether it retains its vasoactivity. Further studies are needed to address this important issue.

**Conclusions**

We used mice overexpressing mutated forms of APP to investigate the relative contribution of plasma and brain Aβ1–40 in the cerebrovascular dysfunction. We found that the cerebrovascular alterations are also present in Tg-SwDI...
mice, which have elevated levels of Aβ1–40 only in brain. However, the dysfunction is less marked than in Tg-2576 mice, in which both plasma and brain Aβ1–40 are increased. Intravascular administration of exogenous Aβ1–40 aggravates the cerebrovascular function in Tg-SwDI, but not Tg-2576 mice. The data indicate that plasma and brain Aβ1–40, acting on distinct targets on opposite sides of the vessels wall, exert additive effects on cerebrovascular regulation, and have implications for clinical conditions in which plasma levels of Aβ are elevated.

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Disclosures

None.

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