Aβ-peptides enhance vasoconstriction in cerebral circulation

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Aβ-peptides enhance vasoconstriction in cerebral circulation. Am J Physiol Heart Circ Physiol 281: H2417-H2424, 2001.—Amyloid-β (Aβ)-peptides are involved in the pathophysiology of Alzheimer’s dementia. We studied the effects of Aβ on selected constrictor responses of cerebral circulation. Mice were anesthetized (by using urethane-chloralose) and equipped with a cranial window. Arterial pressure and blood gases were monitored and controlled. Cerebral blood flow (CBF) was monitored by a laser Doppler probe. Topical superfusion with Aβ1–40 (0.1–10 μM), but not with the reverse peptide Aβ40–1, reduced resting CBF (∆ρ = 4% at 5 μM; P < 0.05) and augmented the reduction in CBF produced by the thromboxane analog U-46619 (+45 ± 3% at 5 μM; P < 0.05). Aβ1–40 or Aβ1–42 did not affect the reduction in CBF produced by hypoxia. The reduction in resting CBF and the enhancement of vasoconstriction were reversed by treatment with the free radical scavengers superoxide dismutase or manganic(I-II)meso-tetrakis(4-benzoic acid)porphyrin. Substitution of the methionine residue in position 35 with norleucine, a mutation that abolishes the ability of Aβ to produce free radicals, abolished its vascular effects. Nanomolar concentrations of Aβ1–40 constricted isolated pressurized middle cerebral artery segments with intrinsic tone (∆ρ = 3% at 100 nM; P < 0.05). We conclude that Aβ acts directly on cerebral arteries to produce vasoconstriction and to enhance the formation of reactive oxygen species (ROS) in vivo and began to study the mechanisms of this effect. The vascular actions of Aβ may contribute to the deleterious effects resulting from accumulation of this peptide in Alzheimer’s dementia.

Alzheimer’s disease; cerebral blood flow; reactive oxygen species; laser Doppler flowmetry

STUDIES OVER THE PAST two decades have indicated that the amyloid precursor protein (APP) and peptides derived from its processing are involved in the pathogenesis of Alzheimer’s dementia (AD) (for a review, see Ref. 24). Thus mutations of the APP gene are linked to certain familial forms of AD (14), and amyloid-β (Aβ), a peptide produced by proteolytic processing of APP, is a major component of the amyloid plaques present in the brain of patients with AD (4). In addition, overexpression of mutated APP in transgenic mice increases Aβ concentration in the brain and leads to formation of amyloid plaques and cognitive impairment, both features characteristic of AD (21). Thus Aβ-peptides seem to play a crucial role in the brain dysfunction associated with AD.

Mechanisms by which Aβ exerts its pathogenic effects have not been fully elucidated (for a review, see Ref. 15). Recent evidence suggests that Aβ in addition to its well-known neurotoxicity impairs the function of the cerebral circulation (11). Transgenic mice overexpressing APP and Aβ exhibit a profound attenuation in the increase of cerebral blood flow (CBF) produced by endothelium-dependent vasodilators or by neural activation (11, 18). These effects are also observed after application of synthetic Aβ to the cerebral cortex of normal mice and are counteracted by free radical scavengers (17).

Much less is known about the influence of Aβ on constrictor responses of the cerebral circulation. Although previous studies have investigated the constrictor effect of Aβ on isolated arteries, these studies had limitations related to the use of pharmacologically preconstricted isolated vessels (22, 28, 29). Therefore, in the present study, we investigated the effect of synthetic Aβ on constrictor responses of the cerebral microcirculation in vivo and began to study the mechanisms of the effect. We found that topical application of Aβ to the mouse neocortex reduces resting CBF and enhances the reduction in CBF produced by the thromboxane analog U-46619. These effects are reversed by free radical scavengers and do not occur when a mutated form of Aβ that does not produce reactive oxygen species (ROS) is used. Furthermore, Aβ produces constriction in isolated-pressurized mouse middle cerebral arteries with intrinsic tone. The findings provide evidence that Aβ-peptides render the cerebral circulation more sensitive to certain vasoconstrictors. The result-
ing oligemia may contribute to brain dysfunction in conditions associated with Aβ accumulation in the brain.

**METHODS**

Methods for surgical preparation of mice, for topical application of drugs, and for monitoring CBF using laser Doppler flowmetry have been described in detail in previous publications (11, 16, 17) and are briefly summarized below.

**General Surgical Procedures**

Studies were conducted in 53 C57BL/6J male mice (age 2–3 mo, body wt 20–30 g) obtained from Jackson Laboratories (Bar Harbor, ME). Mice were anesthetized with halothane in 100% O2 (induction 5%; maintenance 1–2%). Trachea were intubated and mice were artificially ventilated connected to a heating lamp. End-tidal CO2, monitored by a CO2 analyzer (Capstar-100, CWE), was maintained at 2.6 to 2.7% (Table 1). After surgery, halothane was discontinued and anesthesia was maintained with urethane (750 mg/kg ip) and chloralose (50 mg/kg ip). Throughout the experiment, the level of anesthesia was monitored by testing corneal reflexes and motor responses to tail pinch. To minimize confounding effects of anesthesia on vascular reactivity, the time interval between the administration of urethane-chloralose and testing of CBF responses was kept consistent among the different groups of mice studied.

**Monitoring CBF**

A small craniotomy (2 × 2 mm) was performed to expose the parietal cortex, the dura was removed, and the site was superfused with Ringer solution (37°C; pH 7.3–7.4) (16). CBF was continuously monitored at the site of superfusion with a laser Doppler probe (Vasamedic; St. Paul, MN) positioned stereotaxically on the cortical surface. CBF values were expressed as percent increase relative to the resting level. Zero values for CBF were obtained after the heart was stopped by an overdose of halothane at the end of the experiment. Although laser Doppler flowmetry is not quantitative, it monitors relative changes in CBF quite accurately (see Ref. 10 for a review).

### Table 1. Mean arterial pressure and blood gases in the mice studied

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>PaCO2, mmHg</th>
<th>PaO2, mmHg</th>
<th>pH</th>
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<tr>
<td><strong>Effect of Aβ on response to U-46619 and hypocapnia</strong></td>
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<tr>
<td>U-46619 Ringer</td>
<td>96 ± 2</td>
<td>34.3 ± 1.0</td>
<td>131 ± 7</td>
<td>7.33 ± 0.01</td>
<td>6†</td>
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<tr>
<td>Aβ1–40</td>
<td>92 ± 4</td>
<td>35.0 ± 1.1</td>
<td>129 ± 5</td>
<td>7.32 ± 0.01</td>
<td>6†</td>
</tr>
<tr>
<td>Aβ1–42</td>
<td>95 ± 4</td>
<td>33.2 ± 1.5</td>
<td>127 ± 5</td>
<td>7.33 ± 0.01</td>
<td>6†</td>
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<tr>
<td>Aβ1–42 (no DMSO)</td>
<td>95 ± 2</td>
<td>35.7 ± 1.6</td>
<td>127 ± 5</td>
<td>7.31 ± 0.01</td>
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<tr>
<td>Hypocapnia</td>
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<tr>
<td>Aβ1–40</td>
<td>91 ± 3</td>
<td>20.5 ± 0.7ª</td>
<td>130 ± 5</td>
<td>7.45 ± 0.02ª</td>
<td>6†</td>
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<tr>
<td>Aβ40–1</td>
<td>92 ± 4</td>
<td>20.3 ± 0.6ª</td>
<td>131 ± 6</td>
<td>7.48 ± 0.01ª</td>
<td>6†</td>
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<tr>
<td>Aβ1–42</td>
<td>94 ± 2</td>
<td>21.0 ± 0.8ª</td>
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<td><strong>Effect of Aβ±SOD or MnTBAP and of Aβ1–40(M35Nle) on response to U-46619</strong></td>
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<td>Aβ1–40+SOD</td>
<td>91 ± 3</td>
<td>34.5 ± 1.2</td>
<td>119 ± 8</td>
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<tr>
<td>Aβ</td>
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<td>Aβ+SOD</td>
<td>89 ± 3</td>
<td>35.0 ± 1.2</td>
<td>123 ± 9</td>
<td>7.31 ± 0.01</td>
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<tr>
<td>Aβ1–42+SOD</td>
<td>95 ± 2</td>
<td>34.0 ± 0.7</td>
<td>130 ± 5</td>
<td>7.31 ± 0.01</td>
<td>6</td>
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<tr>
<td>MnTBAP</td>
<td>95 ± 2</td>
<td>34.3 ± 1.1</td>
<td>129 ± 5</td>
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<td>MnTBAP</td>
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<td><strong>MnTBAP</strong></td>
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<tr>
<td>Ringer</td>
<td>92 ± 4</td>
<td>34.5 ± 1.2</td>
<td>124 ± 5</td>
<td>7.29 ± 0.01</td>
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<tr>
<td>MnTBAP</td>
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<td>34.4 ± 1.0</td>
<td>131 ± 10</td>
<td>7.29 ± 0.01</td>
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<tr>
<td>SOD</td>
<td>90 ± 4</td>
<td>34.0 ± 1.2</td>
<td>128 ± 7</td>
<td>7.31 ± 0.01</td>
<td>6</td>
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<tr>
<td>Aβ1–40(M53Nle)</td>
<td>91 ± 4</td>
<td>34.3 ± 0.9</td>
<td>135 ± 10</td>
<td>7.30 ± 0.02</td>
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</tr>
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Values are means ± SE. n = no. of mice. MAP, mean arterial pressure; PaCO2 and PaO2, arterial PCO2 and PO2, respectively; Aβ, amyloid-β; SOD, superoxide dismutase; MnTBAP, manganic (I–II) meso-tetrakis (4-benzoic acid) porphyrin; Ringer, Ringer solution. *P < 0.01 from respective control; †U-46619 and hypocapnia were tested in the same mice.
Vascular Diameter in Pressurized Middle Cerebral Arteries

Mice were deeply anesthetized with pentobarbital and decapitated. As described in detail elsewhere (13, 20), the brain was removed and quickly transferred to and kept in a normal physiological salt solution (PSS) composed of (in mM) 119 NaCl, 4.7 KCl, 24.0 NaHCO₃, 1.2 KH₂PO₄, 1.6 CaCl₂, 1.2 MgSO₄, 0.023 EDTA, and 11 glucose. PSS was continuously bubbled with 95% O₂, 5% CO₂, adjusted to pH 7.4 with NaOH at 0–4°C on melting ice. The middle cerebral arteries were dissected from the brain and placed in PSS. The resistance-sized (<150 μm diameter at 10 mmHg) sections (1–2 mm length) of artery were cleaned of connective tissue and cannulated using glass micropipettes. The intact artery segment was secured in place on the pipettes with nylon ties and continuously superfused with PSS at 37°C, at a rate of 3–5 ml/min. After a 20-min equilibration period, intravascular pressure was gradually raised from 10 mmHg to 60 mmHg. Arteries that did not constrict in response to pressure were not used. The artery was viewed through an inverted microscope equipped with a video camera. Vessel lumen diameter was continuously measured with a video dimension analyzer (Living Systems; Burlington, VT), recorded via an analog-to-digital converter (DataQ Instruments; Akron, OH), and stored on disk for off-line analysis. The maximal diameter of each artery was measured by removal of Ca²⁺ from the superfusing PSS. Experiments with Aβ superfusion were performed in an intraluminal pressure of 60 mmHg. Aβ₄₀–40 was dissolved in PSS and superfused on the artery.

Experimental Protocols

Effect of Aβ superfusion on vasoconstrictor responses. After stabilization of MAP and blood gases (Table 1), the thromboxane A₂ analog U-46619 (1 μM, Sigma), a potent vasoconstrictor (e.g., Ref. 6), was superfused on the exposed cerebral cortex until the evoked change in CBF reached a steady state (usually 3–5 min). The superfusion solution was then switched back to normal Ringer solution and CBF returned to baseline. Concentration of U-46619 was chosen in preliminary experiments to produce 50% of maximal responses as determined by dose-response curves (11). The reduction in CBF produced by systemic hypocapnia was also tested. Hypocapnia [arterial PCO₂ (Paco₂) = 18–22 mmHg] was induced by hyperventilation and by reducing CO₂ through the circuit of the ventilator. Hypocapnia was monitored by end-tidal CO₂ and by measuring Paco₂ after the reduction reached a steady state. After responses during Ringer solution superfusion were tested, the superfusion solution was changed to Ringer solution containing increasing concentrations of Aβ₁–40 (0.01–10 μM), Aβ₁–42 (0.01–10 μM), or the control peptide Aβ₁–40 (0.01–10 μM) (Sigma). To minimize aggregation of the peptide during the experiment and to prevent potential effects on aggregation by superoxide dismutase (SOD) or manganic (II) meso-tetrakis (4-benzoic acid) porphyrin (MnTBAP), Aβ was freshly solubilized in DMSO and then diluted in normal Ringer solution. The final DMSO concentration was <0.2%. This concentration of DMSO does not affect resting CBF, does not attenuate the reduction in CBF produced by topical application of U-46619 and hypocapnia, and does not affect vasodilatory responses of the cerebral circulation (unpublished observations; 17, 18, 26, 35). In some studies, to rule out the possibility that the biological activity of Aβ₁–42 was diminished by DMSO, a ROS scavenger, this peptide was dissolved in Ringer solution without DMSO and the constrictor effect of U-46619 was tested. For each Aβ concentration, responses to U-46619 or hypocapnia were tested after 30–40 min of superfusion. This time interval was selected on the basis of preliminary experiments in which the time course of the cerebrovascular effects of Aβ was investigated.

Effect of superoxide scavengers on the cerebrovascular actions of Aβ. The window was superfused with Ringer solution and the effect of U-46619 on CBF was tested. Ringer solution containing Aβ₁–40 (5 μM) was then superfused for 30 min and the effect of U-46619 was tested again. The superfusion solution was then changed to Ringer solution containing Aβ₁–40 and either SOD (Sigma; 100–500 U/ml) or the SOD mimetic MnTBAP (25–100 μM) or MnTBAP (25–100 μM) and the effect of resting CBF and the response to U-46619 were assessed 30 min later. SOD is a superoxide scavenger that, due to its molecular weight, is thought to act on extracellular superoxide (see Ref. 27). MnTBAP is a cell-permeant agent and is thought to scavenge superoxide both intracellularly and extracellularly (2, 7, 12). In some studies, the window was superfused with Ringer solution containing SOD (100 and 500 U/ml) or MnTBAP (25–100 μM) and the effects of resting CBF and the response to U-46619 were assessed 30 min later. Effect of superfusion with Aβ₁–40 (M3S5Nle). In some studies, we investigated the cerebrovascular effects of Aβ₁–40 in which the methionine residue in position 35 was substituted with the norleucine Aβ₁–40 (M3S5Nle), an amino acid structurally similar to methionine but lacking the sulfur atom (32). Such substitution eliminates the ability of Aβ₁–40 to generate ROS (32). Aβ₁–40 (M3S5Nle) (purity >95%; lot number 6640; AnaSpec, San Jose, CA) was dissolved in DMSO and superfused at a concentration of 5 μM. Effects on resting CBF and on cerebrovascular responses evoked by U-46619 were tested after 30 min of superfusion.

Data Analysis

Data in text and figures are expressed as means ± SE. Two-group comparisons were analyzed by the two-tailed t-test for dependent or independent samples as appropriate. Multiple comparisons were evaluated by the analysis of variance and Tukey’s test. The data on isolated middle cerebral arteries were evaluated by the Dunnett’s procedure for comparing multiple treatments with one control. Probability values of <0.05 were considered statistically significant.

RESULTS

Effect of Aβ ON CBF Reductions Produced by U-46619 or Hypocapnia

Aβ₁–40 (0.1–10 μM), but not Aβ₁–42 or the reverse peptide Aβ₄₀–1, reduced resting CBF in a dose-dependent manner (Fig. 1). The reduction in CBF was sustained for at least 2 h after the onset of Aβ₁–40 superfusion. Aβ dose dependently enhanced the reduction in CBF produced by U-46619. The effect was more pronounced for Aβ₁–40 than for Aβ₁–42 and was not observed with Aβ₄₀–1 (Fig. 1). To rule out the possibility that the lower potency of Aβ₁–42 was due to DMSO (the solvent used to dissolve Aβ), we also tested Aβ₁–42 dissolved in Ringer solution. There were no differences between the enhancement of the U-46619-induced constriction produced by Aβ₁–42 dissolved in Ringer solution (−24.4 ± 1.3%) and DMSO (−24.6 ± 1.3%; P > 0.05; n = 6; t-test). In contrast to U-46619, the reduction in CBF produced by hypocapnia was not enhanced by Aβ₁–40 or 1–42 compared with superfusion with Ringer solution or with the inactive peptide Aβ₄₀–1 (Fig. 2).
To confirm that the constrictor effect of Aβ was independent of parenchymal factors, we studied the effect of this peptide on isolated pressurized (60 mmHg) middle cerebral arteries with intrinsic myogenic tone (internal diameter 111 ± 7 μm, n = 7 arteries). Aβ1–40 produced a dose-dependent constriction statistically significant at a concentration of 1 nM and was well developed at 100 nM (Fig. 3; P < 0.05 from control).

**Effect of SOD and MnTBAP on Cerebrovascular Actions of Aβ**

The alterations in endothelium-dependent vasodilation produced by Aβ are counteracted by superoxide-scavenging agents (17). Therefore, we investigated whether the constrictor effects of Aβ1–40 are reversed by the superoxide scavengers SOD or MnTBAP. During Ringer solution superfusion, Aβ1–40 (5 μM) attenuated resting CBF and enhanced constrictor response to U-46619 (Fig. 4). Superfusion with SOD (100–500 U/ml) or MnTBAP (25–100 μM) counteracted the reduction in resting CBF and enhancement of the constrictor effect of U-46619 (Fig. 4). MnTBAP was more effective than SOD (Fig. 5), a finding probably reflecting the better brain penetration of MnTBAP, or the fact that MnTBAP scavenges both superoxide and hydrogen peroxide (2, 7, 12). As for Aβ1–40, the enhancement of the constrictor effect of U-46619 produced by Aβ1–42 was offset by SOD or MnTBAP (Figs. 4 and 5).

![Graph A](image1.png)

**Fig. 1.** Effect of topical superfusion of amyloid-β (Aβ1–40, Aβ40–1, and Aβ1–42 on resting cerebral blood flow (CBF) (A) and on the reduction in CBF produced by 1 μM U-46619 (B). Data were statistically evaluated by ANOVA and Tukey’s test.

![Graph B](image2.png)

**Fig. 2.** Effect of topical superfusion of Aβ1–40, Aβ1–42, and Aβ40–1 on the reduction in CBF produced by hypocapnia. Data were statistically evaluated by ANOVA and Tukey’s test.
In the absence of Aβ, SOD (500 U/ml; n = 6) or MnTBAP (100 μM; n = 5) did not influence resting CBF (before SOD 19.1 ± 4.0 and after SOD 19.4 ± 4.1 perfusion units; before MnTBAP 18.3 ± 4.4 and after MnTBAP 18.2 ± 4.4 perfusion units; P > 0.05) or the reduction in CBF produced by U-46619 (before SOD 219.9 ± 3.0 and after SOD −20.0 ± 2.8%; before MnTBAP −18.7 ± 3.7% and after MnTBAP −19.0 ± 1.3%; P > 0.05; paired t-test). These observations, in conjunction with previous observations (17), indicate that SOD and MnTBAP are devoid of cerebrovascular effects that could confound the interpretation of the results.

Substitution of methionine 35 with the structurally similar amino acid norleucine attenuates the ability of Aβ to generate ROS (32). Therefore, we used Aβ1–40(M35Nle) to provide additional evidence in support of the hypothesis that the constrictor effects of Aβ are mediated by ROS. Aβ1–40(M35Nle) (5 μM) did not reduce resting CBF (before 19.1 ± 3.4 or after 19.2 ± 3.6 perfusion units; P > 0.05; n = 5; paired t-test) and did not alter the reduction in CBF produced by U-46619 (before 219.9 ± 3.0 and after 20.0 ± 2.8%; P > 0.05; paired t-test). These observations, in conjunction with previous observations (17), indicate that SOD and MnTBAP are devoid of cerebrovascular effects that could confound the interpretation of the results.
U-46619 (before \(-18.4 \pm 4.1\) or after \(-19.3 \pm 2.9\%\); \(P > 0.05\); \(n = 5\); paired \(t\)-test).

**DISCUSSION**

We have demonstrated that Aβ peptides attenuate resting CBF and enhance the reduction in CBF produced by the thromboxane analog U-46619 but not by hypocapnia. The enhancement of constriction was more pronounced for Aβ1–40 than for Aβ1–42, and was not observed with the reverse peptide Aβ40–1. Furthermore, Aβ1–40 constricted isolated-pressurized middle cerebral arteries, indicating that the constrictor effects of the peptide are independent of parenchymal mechanisms of this vascular effect of Aβ. We then sought to study the mechanisms of this vascular effect of Aβ with respect to the role of ROS. We found that the free radical scavengers SOD and MnTBAP counteract the Aβ-induced reduction in resting CBF and enhancement of constriction. Furthermore, a mutated form of Aβ that does not generate ROS (32) was devoid of effects on CBF and did not alter constriction. These observations provide strong evidence that the constrictor effects of Aβ peptides are mediated through production of ROS.

Although it has been shown that Aβ produces constriction of systemic and cerebral vessels (22, 28, 29), in these studies, arteries were preconstricted with phenylephrine or serotonin (22, 28, 29), and pharmacological interactions between Aβ and the drug used to produce constriction could not be ruled out. In the present study, by using a cranial window preparation, we were able to demonstrate that the Aβ-induced enhancement of constriction is also observed in the intact cerebral circulation in vivo. Furthermore, we found that Aβ is a potent vasoconstrictor also in isolated-pressurized vessels with intrinsic tone. Constrictor effects were observed at concentrations smaller than those reported to be effective in pharmacologically preconstricted arteries (22, 28, 29). Therefore, the constrictor effects of Aβ seem to be more potent in arteries with intrinsic tone.

It is unlikely that the enhancement of constriction produced by Aβ is due to nonspecific effects of the peptide on all constrictor responses, because the enhancement is observed only in the CBF reductions produced by U-46619 and not by hypocapnia. Therefore, the effect of Aβ is restricted to specific constrictor responses. It is also unlikely that the effect of Aβ is mediated by endothelial cell destruction, as reported in isolated vessel preparations (22, 28, 29). This is because the effects of Aβ on resting CBF and on the response to U-46619 is abrogated by SOD or MnTBAP, indicating that the Aβ-induced alteration is reversible and, as such, cannot be due to necrosis of endothelial cells. This conclusion is also supported by the observation that, in this preparation, the Aβ-induced attenuation of endothelium-dependent CBF responses is counteracted by SOD or MnTBAP (17). Therefore, the enhancement of constriction is not related to endothelial destruction but rather to a specific vascular dysfunction mediated by Aβ.

The cellular mechanisms of the effect of Aβ on constriction remain to be fully elucidated. The observation that Aβ produced constriction also in isolated pressurized middle cerebral arteries indicates that the brain parenchyma is not needed for this action. Therefore, the effect of Aβ could be mediated by actions on endothelial cells and/or vascular smooth muscles. The finding that Aβ affects endothelium-dependent vasodilation suggests that this peptide alters endothelial cell function (11, 17). Therefore, the increased constriction could be due to loss of vasodilator tone provided by endothelium-derived relaxing factors (for a review, see Ref. 15). If this were the case, one would anticipate that all constrictor responses would be enhanced. However, Aβ did not augment the reduction in CBF produced by hypocapnia. Therefore, it is unlikely that the enhancement of vasoconstriction is due solely to increased constrictor tone resulting from impairment of endothelium-dependent relaxation. On the other hand, Aβ could stimulate the production of endothelium-derived constrictor factors (19, 23, 36). Although we have no data to support or disprove this hypothesis, this possibility seems unlikely, because endothelium removal does not affect Aβ vasoactivity in vitro (1), suggesting that endothelial factors are not involved in the vascular effect of Aβ. Therefore, Aβ could act directly on vascular smooth muscle cells to enhance the constrictor response produced by the U-46619. This possibility seems likely in view of the fact that both in our in vivo and in vitro preparations, Aβ was applied only to the abluminal side of the vessel. However, we cannot rule out the possibility that Aβ also reached the endothelium in our preparation. Therefore, further experiments in which the role of endothelial and smooth muscle cells is studied are required to define the cellular site of action of Aβ.

Irrespective of the cellular target(s) of Aβ, our data suggest that its constrictor effects are mediated through production of ROS. However, the free radical species involved remain to be defined. Aβ is thought to produce ROS through different mechanisms (for a review, see Ref. 15). Whereas Aβ itself generates free radical peptides (9), it may also do so by binding to RAGE receptors on endothelial and smooth muscle cells, and by activating NADPH oxidase (3, 34). In addition to NADPH oxidase, common sources of ROS at the vascular level include cyclooxygenase, xanthine oxidase, nitric oxide synthase, and mitochondrial enzymes (30). Therefore, the sources of ROS are likely to be multiple. It remains to be determined whether ROS are the ultimate mediator of constriction or whether other factors are also involved. There is evidence that calcium channels contribute to Aβ vasoactivity (1). Furthermore, it remains to be established whether, in addition to ROS, receptor-dependent mechanisms also play a role in the constrictor effects of Aβ.

The state of aggregation of Aβ, which is influenced by ROS, has a profound effect on the biological activities of the peptide (9, 15). However, the role of peptide aggregation in Aβ vasoactivity has not been defined. Differences in aggregation could explain the difference
in vasoactivity between Aβ1–40 and Aβ1–42. Furthermore, changes in aggregation produced by substitution of the methionine residue in position 35 with norleucine, a mutation that abolishes the ability of Aβ to generate ROS (32), could play a role in the lack of vasoactivity of Aβ1–40(M35Nle). However, this view is not supported by recent studies indicating that the ability of mutated Aβ to form fibrils is not different from that of native Aβ (31).

We have previously demonstrated that in mice overexpressing APP, the reduction in CBF produced by the thromboxane analog U-46619 is enhanced (11). The enhancement of constriction is not observed in double transgenics overexpressing both APP and SOD, suggesting an involvement of ROS in the effect (11). The results of the present study complement and extend these observations by demonstrating that acute application of exogenous Aβ can account in full for this action. Furthermore, we demonstrated that both the short (Aβ1–40) and the long (Aβ1–42) form of the peptide are involved, although Aβ1–40, which is in higher concentration in APP mice (18), is more potent. The mechanisms for the difference in vascular actions of the two forms of the peptide remain to be elucidated.

An important question concerns whether the concentration of Aβ in the cerebrospinal fluid (CSF) of patients with AD would be sufficient to produce vascular dysfunction. The CSF concentration of Aβ1–40 is not increased, whereas Aβ1–42 is reduced compared with nondemented controls (25). However, Aβ levels are greatly elevated in brain and blood vessels of AD patients, with Aβ1–40 being most abundant in vessels (8, 33). Therefore, Aβ in cerebral and vascular tissues, rather than in CSF, is likely to produce cerebrovascular dysfunction in AD patients.

We conclude that Aβ peptides attenuate resting CBF and enhance the reduction in CBF produced by the vasoconstrictor U-46619, an effect more potent for Aβ1–40 than Aβ1–42. The constrictor effects of Aβ are reversed by the free radical scavengers SOD or MnTBAP. The data are consistent with the hypothesis that Aβ, through the production of ROS, enhances selected constrictor responses of the cerebral circulation. These findings raise the possibility that such enhancement of vasoconstriction could reduce CBF and contribute to brain dysfunction in diseases associated with Aβ accumulation in the brain parenchyma or blood vessels.

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