Amyloid-β Peptides Activate α₁-Adrenergic Cardiovascular Receptors

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Abstract—Alzheimer disease features amyloid-β (Aβ) peptide deposition in brain and blood vessels and is associated with hypertension. Aβ peptide can cause vasoconstriction and endothelial dysfunction. We observed that Aβ peptides exert a chronotropic effect in neonatal cardiomyocytes, similar to α₁-adrenergic receptor autoantibodies that we described earlier. Recently, it was shown that α₁-adrenergic receptor could impair blood–brain flow. We hypothesized that Aβ peptides might elicit a signal transduction pathway in vascular cells, induced by α₁-adrenergic receptor activation. Aβ (25–35) and Aβ (10–35) induced a positive chronotropic effect in the cardiac contraction assay (28.75±1.15 and 29.40±0.98 bpm), which was attenuated by α₁-adrenergic receptor blockers (urapidil, 1.53±1.17 bpm; prazosin, 0.30±0.96 bpm). Both Aβ peptides induced an intracellular calcium release in vascular smooth muscle cells. Chronotropic activity and calcium response elicited by Aβ (25–35) were blocked with peptides corresponding to the first extracellular loop of the α₁-adrenergic receptor. We observed an induction of extracellular-regulated kinase 1/2 phosphorylation by Aβ (25–35) in Chinese hamster ovary cells overexpressing α₁-adrenergic receptor, vascular smooth muscle cells, and cardiomyocytes. We generated an activation-state–sensitive α₁-adrenergic receptor antibody and visualized activation of the α₁-adrenergic receptor by Aβ peptide. Aβ (25–35) induces vasoconstriction of mouse aortic rings and in coronary arteries in Langendorff-perfused rat hearts that resulted in decreased coronary flow. Both effects could be reversed by α₁-adrenergic receptor blockade. Our data are relevant to the association between Alzheimer disease and hypertension. They may explain impairment of vascular responses by Aβ and could have therapeutic implications. (Hypertension. 2013;62:00-00.) ● Online Data Supplement

Key Words: α₁-adrenergic receptor ■ Alzheimer disease ■ amyloid-β peptides ■ hypertension ■ signal transduction ■ vasoconstriction

Alzheimer disease (AD) features the accumulation of amyloid-β (Aβ) in neuritic plaques, brain parenchyma, and walls of cerebral vessels.1,2 Aβ peptides are the cleavage products of the transmembrane amyloid precursor protein and are naturally produced within the central nervous system throughout life.3 Aβ are degraded, chemically modified, and cross-linked, thereby increasing their relative insolubility, stability, and toxicity.4 Aβ peptides from amyloid deposits in brain and cerebrospinal fluid vary in amino acid composition, ranging from the full-length Aβ (1–40) and Aβ (1–42) peptides to shorter carboxy-terminal Aβ peptides, as well as aminoterminal truncated species.5 The ability to affect cognitive processes is typical not only of the full-length peptide but also of several Aβ fragments, in particular the undecapeptide Aβ (25–35).6,7 The fragment consists of amino acid residues 25 to 35 in Aβ (GSNKGAIGLM) that can form large β-pleated sheet fibrils similar to those obtained by full-length Aβ. Aβ (25–35) exhibits a potent vasoconstrictor effect in rat skin microvasculature and elicits endothelial dysfunction.8 Aβ (25–35) also induces interleukin 1β release from macrophages and microglia.9

AD may be aggravated by vascular mechanisms.10,11 Aβ peptides can enhance vasoconstriction when deposited in microvessels,12 and cause abnormal vascular reactivity even without vascular deposition.13,14 Epidemiological data associate hypertension with AD.14 In preliminary observations, we observed that several Aβ peptides influence the spontaneously beating rate of neonatal rat cardiomyocytes15 in a fashion similar to autoantibodies we described earlier that stimulate the α₁-adrenergic receptor (α₁-AR) in the brain vasculature.16 Furthermore, Karczewski et al17 used MRI and observed a pathological role of α₁-AR in the brain vasculature. These observations caused us to pursue the mechanisms by which Aβ influences cardiovascular cells.

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Methods

Generation of an Activation State–Sensitive αIA-Adrenergic Receptor Antibody

We generated antibodies directed against the activated form of the αIA-adrenergic receptor (αIA-AR) by adapting an earlier method for other G-protein–coupled receptors.23 Accordingly, the peptide ASDSSNCTQP ( Biosyntan; Berlin, Germany) was chosen from the midportion of the aminoterminal tail of human αIA-AR (aa 8–17; NP_150646, National Center for Biotechnology Information). The peptide was coupled to keyhole limpet hemocyanin (Calbiochem) using the glutaraldehyde method. New Zealand white rabbits were immunized with the conjugated peptide according to standard procedures. Collected antisera were affinity-purified onto the same peptide immobilized to ω-aminohexyl agarose (Sigma-Aldrich).

Cardiac Contraction Assay, Preparation of Human αIA-AR Antibody IgG Fractions

Isolation and cultivation of neonatal heart cells were performed as described previously.24 For detection of chronotropic activity of various Aβ peptides, Aβ (1–15), Aβ (25–35), Aβ (1–40), and Aβ (1–42) obtained from antibodies-online were added to neonatal rat cardiomyocytes at a concentration of 0.1 μmol/L. To show activation of a G-protein–coupled receptor and identify the receptor, β1 (bisoprolol, 1 μmol/L) and β2 (ICI 118,551, 0.1 μmol/L) adrenergic receptor antagonist, angiotensin II receptor type 1 (AT1) receptor blocker, 1 μmol/L irbesartan, αIA-AR blockers, 1 μmol/L prazosin, and 1 μmol/L urapidil were added. For the neutralization experiments, synthetic peptides, P37-LGYWF8, P38-WAFGRV, P39-GRV/FCN, P40-APEDET ( Biosyntan), corresponding to the sequence of the first extracellular loop (P37, P38, P39) and the second extracellular loop (P40) of the human αIA-AR, were each added with a 2000-fold excess. The isolation of immunoglobulin fractions containing human αIA-AR antibodies against the first extracellular loop from serum samples was completed as described earlier.25 The immunoglobulin fractions were added to neonatal rat cardiomyocytes at a dilution of 1:40. The beating rate was counted for 15 seconds and 5, 60, and 240 minutes after the addition of Aβ peptides, antagonists, αIA-AR antibodies, or synthetic peptides corresponding to the sequence of αIA-AR.

Isolation of Adult Rat Ventricular Cardiomyocytes

Single ventricular myocytes were isolated enzymatically from adult male Sprague-Dawley rats (∼250 g) as described in Lamounier-Zepter et al.26 Cardiomyocytes were seeded on laminin-coated slides for confocal microscopy and treated with 1 μmol/L phenylephrine and 0.1 μmol/L of various Aβ peptides for 5 minutes, respectively.

Cell Culture, Western Blot, Confocal Microscopy, and Calcium Measurements

Chinese hamster ovary (CHO) cells and CHO cells stably expressing the human αIA-AR were cultured in F12 HAM medium supplemented with glutamine, 10% FCS, and 1% penicillin/streptomycin. Rat vascular smooth muscle cells (VSMCs) were cultured in SmBM (Lonza) medium supplemented with SmGM-2 Single Quots (Lonza), and 1% penicillin/streptomycin. For determination of extracellular-regulated kinase (ERK) 1/2 phosphorylation, cells were treated with 1 μmol/L phenylephrine and 0.1 μmol/L of various Aβ peptides, respectively. For inhibition experiments, 1 μmol/L of urapidil was added. Western blot experiments were performed as described earlier.26 ERK1/2- and αIA-AR activity in different cell types was detected by immunofluorescence with specific antibodies against phosphorylated ERK (9101, Cell Signaling, 1:100) and the activated αIA-AR (88 μg/mL; 1:25) and then exposed to the secondary antibody (Alexa-488–conjugated antirabbit IgG, Life Technologies, 1:200) by immunofluorescence. For microscopy, the preparation was mounted with mounting medium (Aqua Polymount, Polyscience) under a glass coverslip and analyzed with a Nikon Diaphot microscope. A confocal imaging system (MRC 1024 Bio-Rad, Munich, Germany) with an ultraviolet source and an argon/krypton laser was used. At least 30 cells from each of ≥3 independent experiments were examined under each experimental condition. For each set of experiments, identical settings for power of the light source, confocal aperture, gain, and black level were used. Immunofluorescence was evaluated by confocal microscopy by using pseudocolors to encode fluorescence intensity.

Intracellular calcium levels in rat VSMCs were measured with the Fluo-4 Direct Calcium Assay Kit (Life Technology) according to the manufacturer protocol. Measurements started after the addition of 1 μmol/L phenylephrine, 0.1 μmol/L of various Aβ peptides or 2 μg/mL αIA-AR-autoantibody, respectively. Human IgG containing activating αIA-AR antibodies were obtained from patients requiring ≥2 medication classes to achieve goal control blood pressure values as described earlier.26 For the identification of receptor-binding sites, various synthetic peptides corresponding to the sequence of the first extracellular loop (P37, P38, P39), and the second extracellular loop (P40) of the human αIA-AR were preincubated with a 100-fold excess with 0.1 μmol/L Aβ (25–35) for 30 minutes at 4°C and added, respectively.

Ex Vivo Vascular Activity Analyses

All procedures were performed according to guidelines from the American Physiological Society after appropriate approval. Male wild-type C57BL/6 mice (25–30 g, 8–12 weeks) were used for the measurements of isometric contractions and were euthanized under isoflurane. Mouse thoracic aortas were removed, quickly transferred to cold (4°C), oxygenated (95% O2/5% CO2) Krebs–Henseleit buffer solution, and dissected into 2-mm rings whereby perivascular fat and connective tissue were removed. Rings were incubated in medium 199 supplemented with 1% penicillin/streptomycin and kept at 37°C for 24 hours in the presence or absence of 1 μmol/L Aβ (25–35). For inhibition experiments, 1 μmol/L of prazosin was added. Each ring was positioned between 2 stainless steel wires (diameter, 0.0394 mm) in a 5-mL organ bath of a Multivy Small Vessel Myograph (DMT 610 M; Danish Myo Technology, Denmark).27 The organ bath was filled with Krebs–Henseleit buffer solution. The bath solution was continuously oxygenated with a gas mixture of 95% O2 and 5% CO2, and kept at 37°C (pH 7.4). The rings were placed under tension of 0.3 g.28 The software Chart5 (AD Instruments Ltd, Spechbach, Germany) was used for data acquisition and display. The rings were precontracted with 60 mmol/L KCl and equilibrated until a stable resting tension was acquired. Vessels were exposed to increasing cumulative doses of 3-hydroxytryptamine (3–1000 mmol/L). Drugs were added to the bath solution if not indicated otherwise. Tension is expressed as tension/length (mN/mm).

Male Sprague-Dawley rats (250 g, 11 weeks) were injected intraperitoneally, with 200 I.U. of Heparin-Na in PBS, and anesthetized with 20 mg Pentobarbital in 1 mL distilled water. After complete narcosis was reached, they were euthanized and hearts were rapidly excised. After placing them in ice-cold modified Krebs–Henseleit buffer, the aorta was cannulated. The mounted hearts were perfused in a retrograde fashion at constant pressure (70 mm Hg) with continuously aerated (95% O2/5% CO2) modified Krebs–Henseleit buffer containing (in mmol/L) 118 NaCl, 4.7 KCl, 1.2 MgSO4, 1.8 CaCl2, 22.45 NaHCO3, 0.23 KH2PO4, and 11.1 glucose, at a temperature of 37°C. The hearts were stabilized for 15 minutes and then treated for 20 minutes with 0.1 μmol/L urapidil or distilled water followed by 25 minutes of perfusion with 0.1 μmol/L Aβ (25–35) or distilled water as control. Hugo Sachs Electronic software was used for the data acquisition.

Statistical Analysis

Data are presented as mean±SEM. Group differences were analyzed by t test, Mann–Whitney U test, or ANOVA with Bonferroni post hoc test, as appropriate. A value of P<0.05 was considered statistically significant.

Results

Aβ (25–35) increased the beating rate of neonatal rat cardiomyocytes in a time-dependent manner, whereas the negative control peptide with a reverse sequence of Aβ (25–35) did not change the frequency of spontaneously beating neonatal cardiomyocytes.
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We found that the beating rate increased ≤ 4 hours (\(P \leq 0.005\)). The chronotropic effect of Aβ (25–35) was blocked by prazosin and urapidil, whereas \(\beta_1\) and \(\beta_2\) adrenergic receptor antagonists and angiotensin II AT1 receptor blocker had no effect (Figure 1B). Dose–response curves of Aβ (25–35) were generated (10\(^{-6}\) to 10\(^{-10}\) mol/L) in one log order concentrations and showed a typical sigmoid curve (Figure 1C). The efficacy (E\(\text{max}\)) and potency (EC\(50\)) parameters are derived from these data. The maximal chronotropic effect (E\(\text{max}\)) of Aβ (25–35) was 30.82±1.22 increase in number of bpm, and the EC\(50\) value for Aβ (25–35) was 7.4±1.7 nmol/L. A maximal stimulatory effect was observed at a concentration of 1 \(\mu\)mol/L of Aβ (25–35).

Figure 1. A, Incubation of spontaneously beating neonatal rat cardiomyocytes with amyloid-β (Aβ; 25–35) induced a positive chronotropic effect in a time-dependent manner compared with untreated cardiomyocytes as control. The beating rate increased ≤ 4 hours after long-term incubation. There was no change in the frequency of spontaneously beating neonatal cardiomyocytes in the presence of a negative control peptide with a reverse sequence (Aβ rev.) of Aβ (25–35). B, Influence of different antagonists on the positive chronotropic effect of Aβ (25–35) after 240 minutes (bisoprolol, 1 \(\mu\)mol/L; ICI, 0.1 \(\mu\)mol/L; irbesartan, 1 \(\mu\)mol/L; prazosin, 1 \(\mu\)mol/L; and urapidil, 1 \(\mu\)mol/L). The positive chronotropic effect of Aβ (25–35) was abolished in the presence of \(\alpha_1\)-adrenergic antagonists prazosin and urapidil. C, Dose–response curves of Aβ (25–35) were generated (10\(^{-6}\) to 10\(^{-10}\) mol/L) in one log order concentrations. Dose–response curve for the effect of Aβ (25–35) in the cardiac contraction assay showed a typical sigmoid curve. The efficacy (E\(\text{max}\)) and potency (EC\(50\)) parameters of Aβ (25–35) are derived from these data. D, Aβ peptides exert positive or negative chronotropic effects. Although Aβ (25–35) and Aβ (10–35) increased the beating rate, peptides Aβ (1–15), Aβ (1–40), and Aβ (1–42) decreased beating rate compared with spontaneously beating neonatal rat cardiomyocytes as control. E, Amino acid sequence of Aβ peptides. F, Intracellular calcium mobilization 100 seconds after stimulation. Addition of Aβ (25–35) to vascular smooth muscle cells (VSMCs) led to a calcium response similar to phenylephrine and \(\alpha_1\)-adrenergic receptor \((\alpha_1\text{-AR})\) autoantibody stimulation. Aβ (10–35) resulted in a weaker intracellular calcium release compared with phenylephrine, whereas Aβ (1–15), Aβ (1–40), and Aβ (1–42) showed no calcium mobilization. Measurements are given in relative fluorescent units (RFU). ΔRFU were calculated relative to the untreated control (maximum response minus the minimum response).
Using various Aβ peptides (Figure 1D) in cardiac contraction assay, we observed positive or negative chronotropic activity depending on the amino acid sequence of Aβ peptides (Figure 1E). Although Aβ (25–35) and Aβ (10–35) increased the cardiomyocyte contraction rate, peptides Aβ (1–15), Aβ (1–40), and Aβ (1–42) led to decreased beating rate (P<0.001).

Treatment of rat VSMCs with phenylephrine, Aβ (25–35), Aβ (10–35), and α1-AR autoantibodies led to an immediate increase in intracellular Ca2+, whereas Aβ (1–15), Aβ (1–40), and Aβ (1–42) resembled untreated VSMCs. Aβ (25–35) induced a similar increase in intracellular Ca2+ level to that observed by the stimulation with phenylephrine and α1-AR antibodies (Figure 1E). Aβ (10–35) resulted in a weaker intracellular calcium release compared with Aβ (25–35). For mapping of the binding site of Aβ(25–25) to α1-AR, we used synthetic peptides corresponding to the sequence of the first extracellular loop (P37, P38, P39), and the second extracellular loop (P40) of the human α1-AR (Figure 2A). Epitope α1-AR mapping by cardiac contraction assay was performed with overlapping peptide sequences (Figure 2B). 

As expected, there was a significant effect of concentration on responses in all middle cerebral artery segments. Incubation with Aβ (25–35) significantly enhanced the middle cerebral artery constriction rate response to α1-AR activating antibodies (Figure 2C). In adult cardiomyocytes and VSMCs, Aβ (25–35) elicited ERK signal similar to phenylephrine, whereas all other Aβ peptides induced minor or no ERK activation (Figure 3C).

Figure 2. A, Mapping of the first and second extracellular loops of the α1 adrenergic receptor (α1-AR; P37-LGYWAF, P38-WAFGRV, P39-GRVFCN, P40-APEDET), B, Influence of peptides from the first and second extracellular loops of the α1-AR on the positive chronotropic effect of amyloid-β [Aβ; (25–35)] and α1-AR antibodies in spontaneously beating neonatal cardiomyocytes. Peptides P38 and P39 inhibited the chronotropic activity of Aβ (25–35) and α1-AR antibodies, whereas peptides P37 and P40 had no effect. C, Influence of peptides against the first and second extracellular loops of the α1-AR on intracellular calcium release in vascular smooth muscle cells triggered by Aβ (25–35). Peptide P38 and P39 inhibited intracellular calcium release, whereas P37 had no effect. RFU indicates relative fluorescent units.

Discussion
We found that Aβ peptides can activate the α1-AR in a fashion similar to α1-AR activating antibodies that we described earlier.16 The activation was robust, and the binding site on α1-AR was specifically identified. We showed that the activation could higher (P≤0.005) coronary flow compared with Aβ (25–35) perfused hearts without urapidil pretreatment. Similar results were achieved in middle cerebral artery segments (Figure S2). As expected, there was a significant effect of concentration on responses in all middle cerebral artery segments. Incubation with Aβ (25–35) significantly enhanced the middle cerebral artery response to α1 agonist phenylephrine (P<0.0003) but not to KCl.

We next examined the effects of Aβ peptides on ERK 1/2 signal transduction. Using phosphospecific immunoblotting, we observed that phenylephrine and Aβ (25–35) induced ERK 1/2 phosphorylation in α1-AR-harbor CHO cells (Figure 3A). The incubation of VSMCs with various Aβ peptides also resulted in ERK 1/2 phosphorylation (Figure 3A, right). In situ imaging of ERK 1/2 signaling was performed (Figure 3B and 3C). Aβ (25–35) induced ERK activation in a time-dependent manner in neonatal cardiomyocytes and in α1-AR-harbor CHO cells but not in wild-type CHO cells (Figure 3B). In adult cardiomyocytes and VSMCs, Aβ (25–35) elicited ERK signaling similar to phenylephrine, whereas all other Aβ peptides induced minor or no ERK activation (Figure 3C).

We next generated an activation-state–sensitive α1-AR antibody and confirmed α1-AR activation by Aβ (25–35) and Aβ (10–35; Figure 4A). This conformational-dependent antibody did not react with the nonstimulated α1-AR (Figure 4A). However, when the α1-AR was activated by phenylephrine, we obtained positive staining. This staining could be blocked by urapidil. Aβ (25–35) and Aβ (10–35) induced strong α1-AR staining that was also blocked by urapidil. None of the other Aβ peptides led to receptor activation (data not shown).

The influence of Aβ (25–35) on vasoconstriction of mouse aortic rings was studied in the presence and absence of prazosin. The vasoconstriction to 5-hydroxytryptamine was significantly increased (P≤0.001) in aortic rings by Aβ (25–35) pretreatment (Figure 4B, left). This vasoactive effect was abolished in the additional presence of prazosin during the Aβ (25–35) pretreatment. In a further ex vivo model of Langendorff-perfused rat hearts, Aβ (25–35) induced vasoconstriction of coronary arteries that resulted in decreased coronary flow (Figure 4C). Rat hearts preincubated for 20 minutes with urapidil showed a significant
Figure 3. A. Extracellular-regulated kinase (ERK) 1/2 was activated by amyloid-β (Aβ) peptides in Chinese hamster ovary (CHO) cells, overexpressing the α1-adrenergic receptor (α1-AR-CHO; left) and vascular smooth muscle cells (VSMCs; right) detected by immunoblotting. Cells were treated for 15 minutes with 1 μmol/L phenylephrine and 0.1 μmol/L of different Aβ peptides, respectively. Lane 1 represents untreated cells. Eukaryotic initiation factor 4E (eIF4E) was used as loading control (representative of 3 independent experiments). Relative density measurements provided quantification (Image J). B, Aβ (25–35) induced ERK 1/2 phosphorylation in α1-AR-CHO and neonatal rat cardiomyocytes in a time-dependent manner but not in CHO wild-type cells. CHO wild-type, α1-AR-CHO, and neonatal rat cardiomyocytes cells were treated for 5, 30, and 180 minutes with 0.1 μmol/L of Aβ (25–35), respectively. ERK 1/2 activation was determined using a phosphospecific ERK 1/2 antibody. Immunofluorescence was evaluated using pseudocolors to encode fluorescence intensity. Yellow shows ERK 1/2 activation; red is more intense activation. Cells treated with phenylephrine and Aβ (25–35) showed a strong ERK 1/2 activation. Cells were treated for 5 minutes with 1 μmol/L phenylephrine and 0.1 μmol/L of different Aβ peptides, respectively. Aβ rev. was used as negative control peptide (representative of 3 independent experiments).

Aβ has been identified in media and adventitia of small arteries and capillaries of cerebral and peripheral vessels earlier.11,13,21 Investigators have proposed that vascular dysfunction is an early component of AD pathology.11,13,21,22 In vitro data showed that soluble Aβ can cause abnormal vascular reactivity of peripheral arteries in the absence of vascular deposition or vessel wall dysfunction.2 Amyloid-β-induced vascular dysfunction might be an early step in Aβ diseases and could even precede significant Aβ deposition. Transgenic mice overexpressing amyloid precursor protein show attenuated cerebrovascular reactivity to endothelium-dependent

be relevant because calcium signaling and ERK phosphorylation occurred in VSMCs. Using an activation-state–sensitive α1-AR antibody, we could confirm activation of the α1-AR by Amyloidβ. Aβ (25–35) induced vasoconstriction of mouse aortic rings and in coronary arteries in Langendorff-perfused rat hearts that resulted in decreased coronary flow. Both effects could be reversed by α1-AR blockade. Our data extend the effects of Aβ peptides to the vasculature outside the brain in a functional and structural fashion and could in part explain how Aβ can adversely influence blood vessel function.2

Aβ has been identified in media and adventitia of small arteries and capillaries of cerebral and peripheral vessels earlier.11,13,21 It has been shown by several groups that soluble Aβ can cause abnormal vascular reactivity of peripheral arteries in the absence of vascular deposition or vessel wall dysfunction.12,21 We suggest that α1-AR activation could play a role in this process. Surprisingly the kinetics of the α1-AR activation by Aβ (25–35) is quite distinct from that of natural ligands. The α1-AR activation elicited by Aβ (25–35) lasts for hours, whereas the effects of conventional agonists are much shorter.

It has been shown by several groups that soluble Aβ can cause abnormal vascular reactivity.11,12 Amyloid-β–induced vascular dysfunction might be an early step in Aβ diseases and could even precede significant Aβ deposition. Transgenic mice overexpressing amyloid precursor protein show attenuated cerebrovascular reactivity to endothelium-dependent
vasodilators.11,22 We show for 3 different vascular beds, namely middle cerebral artery, aorta, and coronary arteries that application of exogenous Aβ(25–35) to normal blood vessels ex vivo causes endothelium-dependent vasoconstriction, which can be blocked by α₁-adrenoceptor blockers.

Acute application of Aβ peptide to the cerebral cortex of normal mice reproduces the cerebrovascular effects observed in transgenic mice, linking the Aβ peptide to the mechanisms of the vascular dysfunction.23 Using the apolipoprotein E-deficient mouse model for atherosclerosis, Van de Parre et al showed an involvement of Aβ in cardiovascular disease. Amyloid precursor protein is upregulated in atherosclerosis-prone regions of aorta and intensified atherosclerosis.25,26 We observed amyloid deposits in human atherosclerotic plaques suggesting a link between Aβ and vascular dysfunction beyond their role in the central nervous system.

The Honolulu Heart Program/Honolulu Asia Aging Study showed that the Aβ-related risk for AD was increased when blood pressure was higher.27 The authors postulated that hypertension could compromise vascular integrity leading to cerebral amyloid angiopathy and impaired Aβ clearance from the brain.

Recently, several research groups demonstrated Alzheimer pathology in 2 established mouse models of hypertension.15,25,28 Using transverse aortic constriction and angiotensin II infusion, cerebral amyloid deposition was observed as early as 4 weeks after the insult in both models.15 They observed an increased permeability of the blood–brain barrier with albumin leakage in the cortex. In several brain areas controlling cognitive functions, such as the cortex, soluble oligomers and intermediate amyloids were recognized. These data indicated that increased blood pressure levels can be mechanistically related to the pathophysiology of AD.28 They implicated the receptor for advanced glycation end products in the process.24 Several mouse models for AD have been established and characterized; however, it is unclear whether they develop hypertension or hypertension-induced end-organ damage. Moreover, passive transfer of Aβ protein in vivo will be important to show in vivo relevance.24

In the process of AD, Aβ peptides are degraded and modified. Shorter carboxy-terminal truncated Aβ peptides are generated from the full-length Aβ(1–42) and Aβ(1–40) peptides. These truncated Aβ peptides are more toxic and insoluble.30 From our experiments, we cannot differentiate whether the observed effects leading to impairment of blood vessel function are mediated by paracrine effect or by changing into an aggregated, insoluble form. In AD, the fragment consisting of residues 25 to 35 (GSNKGAIGILM) has been shown to form large sheet fibrils, essentially similar to those obtained by the full-length Aβ.31 Thus, Aβ(25–35) is a disease-related short peptide that represents the actual biologically active region of Aβ, Aβ(25–35) is therefore particularly useful in studying amyloid formation and elongation.31 Aβ(25–35) show potent vasoconstrictor effect in microvasculature and blunts the vasodilator

Figure 4. A. Activation of α₁ adrenergic receptor (α₁-AR) by amyloid-β (Aβ) peptides in adult rat cardiomyocytes and vascular smooth muscle cells (VSMCs) was detected by confocal microscopy using an activation-state-sensitive α₁-AR antibody. Phenylephrine, Aβ(25–35) and Aβ (10–35) activated α₁-AR. Cells were treated for 5 minutes with 1 μmol/L phenylephrine and 0.1 μmol/L of different Aβ peptides, respectively. Immunofluorescence was evaluated using pseudocolors to encode fluorescence intensity. Yellow shows α₁-AR activation; red is more intense activation. B. Vascular response of mouse aorta to cumulative concentrations of 5-hydroxytryptamine (5-HT) after preincubation for 24 hours with vehicle (control), Aβ, prazosin as well as Aβ and prazosin (both at 1 μmol/L). After 24-hour incubation, concentration contraction curve to 5-HT was slightly but significantly increased (P≤0.005) in the presence of Aβ compared with control (left). This vasoconstriction was abolished in the additional presence of prazosin (right). Tension is expressed as tension/length (mN/mm). C. Effects of Aβ(25–35) in the presence and absence of urapidil (both at 0.1 μmol/L) on coronary flow in the rat Langendorff heart. Langendorff-perfused rat hearts subjected 20 minutes with urapidil or distilled water followed by 25 minutes perfusion with Aβ(25–35) or distilled water. The effects of distilled water (control green), Aβ(25–35; red), and Aβ(25–35) in the urapidil pretreated Langendorff heart (black) throughout the experiments are shown as mean±SEM of a time period of 5 minutes. The perfusion with Aβ(25–35) led to a reduction of the coronary flow that can be significantly blocked by a pretreatment with urapidil (P≤0.005).
response of substance P and other vasodilators.6,8 The evidence we presented here gives Aβ (25–35) an additional dimension, namely a direct, G-protein receptor–mediated vascular effect.

**Perspectives**

Aβ (25–35) robustly activates the α1-AR in VSMCs by specifically targeting the first extracellular loop of the receptor. This state of affairs could link hypertension and cardiovascular disease to AD pathogenesis. Moreover, the findings could have therapeutic implications and suggest that site-specific blocking α1-AR could have a salutary influence on AD progression beyond blood pressure–lowering effects.

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**Disclosures**

None.

**References**


**Novelty and Significance**

**What Is New?**

- The present study shows for the first time that amyloid-β peptides induce a signal transduction pathway in cardiovascular cells via activation of α1-adrenergic receptor.

**What Is Relevant?**

- The present study provides further evidence of the association between hypertension and Alzheimer disease. Our data provide a mechanism for the observed impairment of blood vessel function by amyloid-β peptides.
Amyloid-\(\beta\) Peptides Activate \(\alpha_1\)-Adrenergic Cardiovascular Receptors
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AMYLOID-β PEPTIDES ACTIVATE α1-ADRENERGIC CARDIOVASCULAR RECEPTORS

Running head: Amyloid-β peptides

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Supplemental methods
Ex vivo vascular activity analyses

Middle cerebral artery segments were isolated from female Sprague Dawley rats (n=6) and incubated in physiological saline solution (PSS in mmol/L – 130 NaCl, 4 KCl, 1.2 MgSO₄, 4 NaHCO₃, 1.8 CaCl₂, 10 HEPES, 1.18 KH₂PO₄, and 6 glucose) with either 0.1µmol/L Aβ (25-35) or 0.1µmol/L of a negative control peptide with a reverse amino acid sequence (Aβ rev.) of Aβ (25-35) for 4h at 37°C. Vessel segments were then cannulated in a dual vessel chamber (Living Systems). After 30 minutes equilibration at 37°C and 75 mmHg intraluminal pressure, a baseline image was captured using a Nikon Eclipse microscope connected to a Roper Scientific camera. Vessel segments were then exposed to 30, 50, and 80 mmol/L KCl. After rinsing three times using PSS, a new baseline image was obtained. Phenylephrine curve was then obtained by addition of 10⁻⁹ to 10⁻⁴ mol/L Phenylephrine diluted in PSS. The data represent the peak response at each concentration. An average of three internal diameters was obtained at each dose using Nikon Elements Imaging Software. The data are presented as a percentage of the baseline internal diameter.
S1: CHO wildtype, α₁-AR-CHO cells and neonatal rat cardiomyocytes were treated for 5, 30 and 180 min with 0.1 µmol/L of a negative control peptide with a reverse amino acid sequence (Aβ rev.) of Aβ (25-35), respectively. ERK1/2 activation was determined using a phosphospecific ERK1/2 antibody. Immunofluorescence was evaluated using pseudocolors to encode fluorescence intensity. Yellow shows ERK 1/2 activation; red is more intense activation. Aβ (rev) induced no ERK 1/2 phosphorylation in CHO wildtype, α₁-AR-CHO cells and neonatal rat cardiomyocytes (representative of one out of three independent experiments).

S2:

**MCA Constriction to KCl**

**MCA Constriction to phenylephrine**
S2: Effect of Aβ (25-35) or Aβ rev. on KCl (left) and phenylephrine (right) induced contractions on middle cerebral artery (MCA) segments. MCA segments were pre-incubated with 0.1 µmol/L Aβ (25-35) or Aβ rev. (negative control peptide) for 4 hours, respectively. Constrictions were expressed as a percentage of the baseline internal diameter, which was taken as 100%. There was a significant effect of concentration on phenylephrine and KCl responses in all MCA segments. However, incubation with Aβ (25-35) significantly enhanced the MCA response only to the α1 agonist phenylephrine (p=0.0003).