Alzheimer's β-amyloid vasoactivity: identification of a novel β-amyloid conformational intermediate

Fiona Crawford[a,*], Claudio Soto[b], Zhiming Suo[a], Chunhong Fang[a], Timothy Parker[a], Asad Sawar[a], Blas Frangione[b], Mike Mullan[a]

[a] *Roskamp Institute, University of South Florida, 3515 E. Fletcher Ave., Tampa, FL 33613, USA*
[b] *Department of Pathology, New York University Medical Center, 550 First Ave., New York, NY 10016, USA*

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Abstract The β-amyloid (Aβ) peptide has previously been shown to enhance phenylephrine or endothelin-1 induced constriction of aortic rings in vitro. The characteristics of Aβ vasoactivity (dose, fragment length, timing) suggest that the mechanism is distinct from Aβ cytotoxicity. To identify which properties of Aβ determine its biological activity on vessels, we investigated a number of Aβ analogues and fragments, individually and in combination, including those that are known to be associated with Alzheimer's disease ($A\beta_{1-42}$) and hereditary cerebral hemorrhage with amyloidosis – Dutch type ($A\beta(22Q)_{1-40}$). The vasoactivity appears to be related to the conformation adopted by the peptide in solution. The β-pleated sheet rich $A\beta_{1-42}$ and $A\beta(22Q)_{1-40}$ were each less vasoactive than the mainly random coil wild type $A\beta_{1-40}$. However, the most vasoactive Aβ peptides were combinations which contain mixtures of random coil and β-sheet structure. The finding that peptides containing low or high levels of β-pleated conformation are less vasoactive than those containing intermediate amounts of this structural motif allows us to propose the existence of a transitional form between random coil and β-pleated that is the vasoactive species of Aβ. This is the first time that Aβ conformational intermediates have been identified and a biological activity associated with them.

Key words: β-Amyloid; Vasoactivity; β-Sheet; Conformation; Alzheimer's disease

1. Introduction

Cerebral amyloid angiopathy (CAA) is increasingly recognized as a common cause of hemorrhage in the elderly and is one of the pathological features of Alzheimer's disease. Deposition of β-amyloid (Aβ) peptide in the adventitia of cerebral vessels progresses to invasion of the media with destruction of the smooth muscle cells of that layer [1]. In a phenotypically closely related condition, hereditary cerebral hemorrhage with amyloidosis – Dutch type (HCHWA-D), both a genetic variant of the Aβ peptide (Aβ22Q) and the wild type Aβ are deposited in the walls of the cerebrovasculature [2]. In both CAA and HCHWA-D the $A\beta_{1-40}$ peptide, rather than the $A\beta_{1-42}$, is the predominant form deposited in vessels [3,4]. In cell culture experiments, Aβ peptides, including $A\beta(22Q)_{1-40}$ are cytotoxic to components of the vessel wall – endothelial and smooth muscle cells [5,6].

We have previously demonstrated Aβ vasoactivity within minutes of application of lower doses of $A\beta_{1-40}$ (50 nM–1 μM) than those used in cytotoxicity experiments. In rings of rat aorta enhancement of phenylephrine or endothelin-1 constriction occurs when freshly solubilized peptide is applied [7,8]. Pre-treatment with freshly solubilized $A\beta_{1-40}$ enhances the vasoconstriction due to physiologic doses of endothelin-1, while $A\beta_{1-42}$ demonstrates a decreased tendency to enhance and no vasoactivity is observed with $A\beta_{25-35}$ [8]. The different degrees to which Aβ peptides of different length, composition and conformational propensity enhance vasoconstriction suggested to us that there may be a relationship between Aβ conformation and vasoactivity. Therefore we extended our observations by testing the activity of other Aβ analogues with different conformational propensities. We also examined the effects of mixing wild type $A\beta_{1-40}$ with either the β-sheet rich Dutch $A\beta(22Q)_{1-40}$ or $A\beta_{1-42}$, and mixing $A\beta_{1-42}$ with Dutch $A\beta(22Q)_{1-40}$.

2. Materials and methods

2.1. Vasoactivity assay

Vasoconstriction was measured in aortic rings from normal male Sprague-Dawley rats using a tissue bath system as previously described [7], and enhancement of constriction induced by the major cerebral vasoregulator endothelin-1 (ET-1). Following a 2 h equilibration we used a range of doses of ET-1 (0.5, 1, 2, 3, 4 and 5 nM) for pre-treatment constriction of the rings. After a further 2 h of equilibration, freshly solubilized peptide was added to the system. The following peptide concentrations were used: 1 μM wild type $A\beta_{1-40}$; 1 μM $A\beta(22Q)_{1-40}$; 1 μM $A\beta_{1-42}$; 0.5 μM wild type $A\beta_{1-40}$ and 0.5 μM $A\beta(22Q)_{1-40}$ mixed; 0.5 μM $A\beta_{1-42}$ and 0.5 μM $A\beta(22Q)_{1-40}$ mixed; 0.5 μM wild type $A\beta_{1-40}$ and 0.5 μM $A\beta_{1-42}$ mixed; 1 μM $A\beta_{25-35}$, 1 μM $A\beta_{22-35}$, 1 μM $A\beta_{1-28}$ or no peptide. The post-treatment contraction was carried out using the same doses of ET-1 as in the pre-contraction. $A\beta_{1-40}$ and $A\beta_{1-42}$ were purchased from MD Enterprise; $A\beta_{22-35}$, $A\beta_{1-28}$ and $A\beta_{25-35}$ from Sigma; and the $A\beta(22Q)_{1-40}$ peptide was obtained from WMKeck Foundation, Yale University. The percentage contraction as compared to baseline was determined for both the pre- and the post-treatment contractions and the difference between post- and pre- was calculated. The mean difference in percentage contraction at each ET-1 dose for each treatment group was then calculated. At each dose of ET-1, all non-zero control values for mean difference in percent contraction were standardized to zero, and the values for other treatment groups were then adjusted accordingly. Unadjusted data were analyzed by ANOVA and by post-hoc comparison of the means using Scheffé's correction for multiple comparisons.

2.2. Circular dichroism (CD) studies

CD spectra were recorded on a Jasco spectropolarimeter Model J-720 at room temperature in a 0.1 cm path-length cell. Stock solutions of Aβ peptides were prepared in 50% acetonitrile and stored lyophilized in aliquots. 50 μg of each peptide was dissolved in 335 μl of 10 mM Tris, pH 7.4. For the experiments involving the combination of two Aβ variants, 25 μg of each peptide was used. CD spectra

*Corresponding author. Fax: (1) (813) 974 3915.
E-mail: fcrawfor@com1.med.usf.edu

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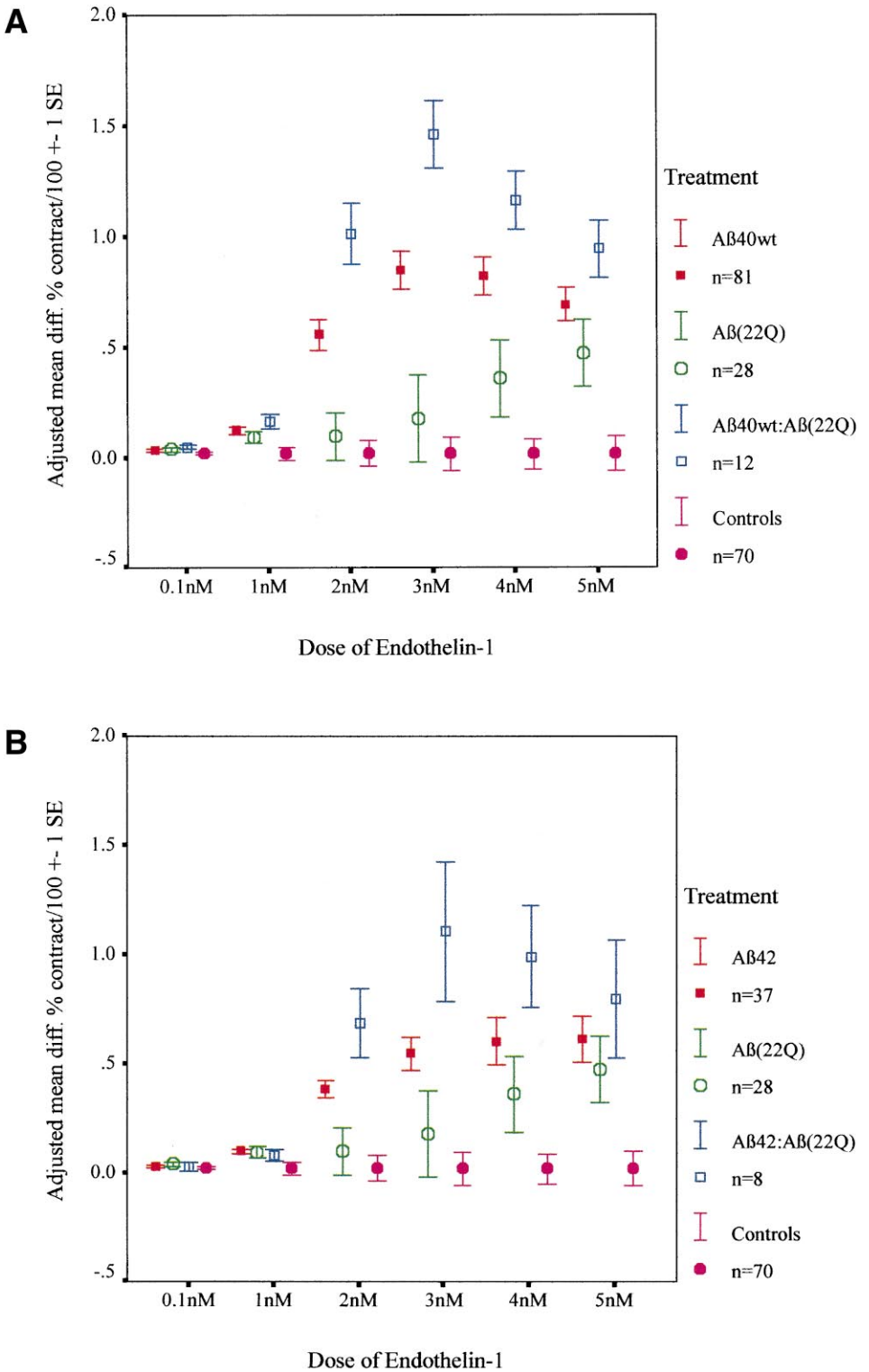

Fig. 1. Vasoactivity of Aβ peptides. Aortic rings were constricted by addition of the doses of endothelin (ET-1) shown. Following equilibration, 1 μM of peptide or peptide combinations were added as indicated and the constriction repeated with the same ET-1 doses. The percentage increase over baseline in the pre-treatment contraction was subtracted from the percentage increase over baseline in the post-treatment contraction and the mean difference for each treatment at each dose was calculated and adjusted values (see text for details) were plotted as shown. Aβ40wt = wild type $Aβ_{1–40}$; Aβ(22Q) = $Aβ(22Q)_{1–40}$; Aβ42 = $Aβ_{1–42}$. A: Enhancement of ET-1 vasoconstriction in the presence of $Aβ_{1–40}$ ($P < 0.001$ compared to controls), lesser enhancement with mutated $Aβ(22Q)_{1–40}$ ($P = 0.03$ compared to controls) and the significantly vasoactive combination of wild type and mutated $Aβ_{1–40}$ ($P = 0.01$ compared to $Aβ_{1–40}$, and $P < 0.001$ compared to $Aβ(22Q)_{1–40}$). B: Similar results with $Aβ_{1–42}$ which produces contractions significantly different from controls ($P < 0.001$) and when combined with $Aβ(22Q)_{1–40}$ is significantly more vasoactive than $Aβ(22Q)_{1–40}$ alone ($P = 0.003$).

were recorded 2 h after solubilization of the peptide and after centrifugation to removed aggregated material. Forty scans were recorded at 0.2 nm intervals over the wavelength range 190–250 nm. Results are expressed in terms of molar ellipticity in units of deg cm^2 $dmol^{-1}$ and the spectra analyzed by the Lincomb algorithm to estimate the percentages of the various secondary structure motifs [9].

3. Results and discussion

The influence of various Aβ analogues on vasoconstriction of rat aortic rings was studied in the presence of ET-1. Wild type $Aβ_{1–40}$ gave the expected enhancement of contraction compared to controls ($P < 0.001$), while mutated $Aβ(22Q)_{1–40}$ was also, to a lesser extent, significantly different from untreated rings ($P = 0.03$). Enhancement of contraction by wild type $Aβ_{1–40}$ was significantly different from that caused by $Aβ(22Q)_{1–40}$ ($P < 0.001$). However, no individuals have been reported who are homozygous for the Dutch mutant, therefore we mixed freshly solubilized wild type $Aβ_{1–40}$ and $Aβ(22Q)_{1–40}$ in a 50:50 proportion at a final concentration of 1 μM. This combination produced enhancement of vasoconstriction greater than that previously noted for any Aβ peptide (Fig. 1A). Enhancement by this mixture was significantly higher than that produced by Aβ(22Q) alone ($P < 0.001$) or by wild type $Aβ_{1–40}$ alone ($P = 0.01$). This prompted us to compare the vasoactivity of other fragments of Aβ of differing amyloidogenic potential individually and in combination. $Aβ_{25–35}$ has previously been reported by us to not enhance ET-1 vasoconstriction [8]. In these experiments, $Aβ_{25–35}$, $Aβ_{22–35}$ and $Aβ_{1–28}$ peptides did not differ significantly from one another, and furthermore were not significantly different from controls (data not shown). $Aβ_{1–42}$ also enhanced vasoactivity as expected compared to untreated rings ($P < 0.001$) [8]. The combination of $Aβ_{1–42}$ and $Aβ(22Q)_{1–40}$ in a 50:50 proportion at final concentration of 1 μM, produced contractions which were significantly higher than Aβ(22Q) alone ($P = 0.003$), and showed a tendency to be higher than $Aβ_{1–42}$ alone (Fig. 1B). Similarly, a 50:50 mixture of $Aβ_{1–40}$ with $Aβ_{1–42}$ also tended to be more vasoactive than $Aβ_{1–42}$ alone (data not shown).

It has been shown that Aβ can adopt different conformations depending on the sequence, length of the peptide and environmental conditions [10]. Aβ can exist in at least two different conformational states, one more random coil/α-helical the other β-pleated [10], and the transition from α-helical/random coil to the β-sheet form is well documented [11–13]. The Aβ conformations differ in several properties including fibrillogenicity, neurotoxicity, resistance to proteolytic degradation and interaction with amyloid-associated proteins [10,11,14,15]. In order to study whether the differences in vasoactivity of various Aβ analogues may have a conformational basis, the secondary structure of the peptides and combinations of peptides was evaluated by CD. As expected from previous experiments [11], $Aβ_{1–40}$ has a mainly unordered structure, while both $Aβ(22Q)_{1–40}$ and $Aβ_{1–42}$ are rich in β-sheet conformation (Fig. 2A). The combination of $Aβ_{1–40}$ with $Aβ(22Q)_{1–40}$ or with $Aβ_{1–42}$, or of $Aβ_{1–42}$ with $Aβ(22Q)_{1–40}$ produced intermediate spectra, indicating a mixture between random coil and β-sheet structures. This result could be interpreted as suggesting that the Aβ peptides did not interact and the resulting CD spectrum corresponds to an average between the spectra of each individual peptide. Alternatively, they may interact producing conformational changes

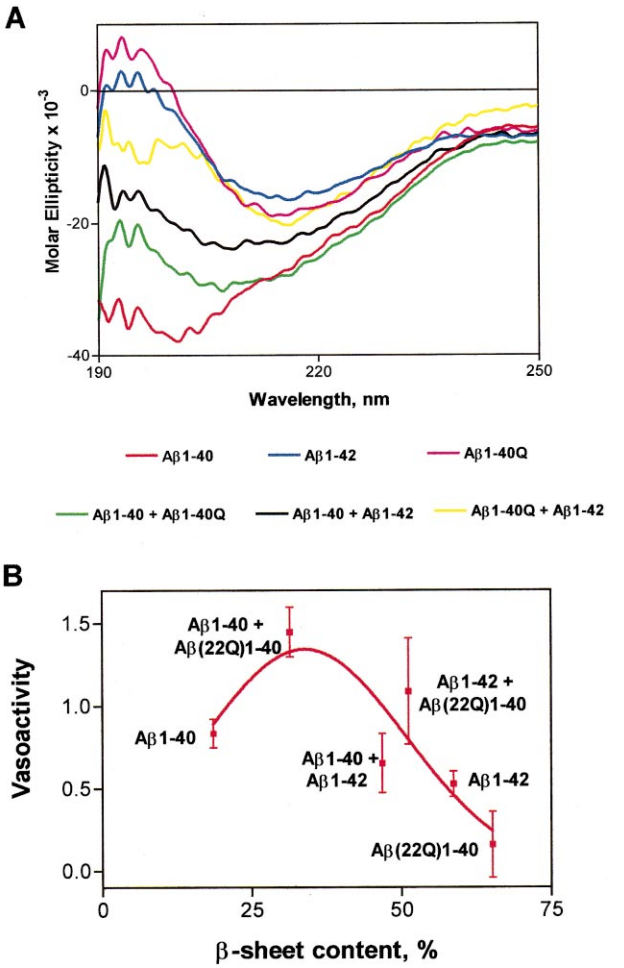

Fig. 2. Relationship between vasoactivity and conformation of Aβ peptides. A: Circular dichroism spectra of different Aβ variants were recorded as described [12]. 50 μg aliquots of each peptide were dissolved in 335 μl of 10 mM Tris, pH 7.4. For the experiments involving the combination of two Aβ variants, 25 μg of each peptide was used. CD spectra were recorded 2 h after solubilization of the peptide, in a Jasco spectropolarimeter, model J-720 at room temperature in a 0.1 cm path-length cell. Forty scans were recorded at 0.2 nm intervals over the wavelength range 190–250 nm. Results are expressed in terms of molar ellipticity in units of deg cm^2 $dmol^{-1}$. B: The percentage of β-sheet content of each peptide and combinations of peptides was estimated by analysis of CD spectra using the Lincomb algorithm [9] and plotted against the percentage of vasoconstriction at 3 nM of endothelin-1, estimated as described in Fig. 1.

that give rise to a structural intermediate composed partially of random coil and partially of β-sheet structure.

The latter explanation is supported by the relationship observed between vasoactivity at the dose of ET-1 which gives greatest separation between different Aβ analogues (3 nM), and the β-sheet content of those same analogues (Fig. 2B). The most vasoactive species was obtained in the combination of $Aβ_{1–40}$ and $Aβ(22Q)_{1–40}$, which contains approximately 30% of β-sheet. If this percentage of β-structure were due to a simple average between the β-sheet contents of the wild type and Dutch peptides, it would be expected that the mixture should produce an intermediate level of vasoconstriction. Likewise the $Aβ_{1–42}$ and $Aβ(22Q)_{1–40}$ combination is significantly more vasoactive, but contains less β-sheet (approximately 50%) than either peptide individually (Fig. 2B).

We investigated the possible contribution of peptide po-

lymerization to Aβ vasoactivity using sedimentation assays as previously described [10], although it has previously been demonstrated that at low concentrations and with short incubation times Aβ can adopt distinct conformations *without* changes in oligomerization stage [11]. Different Aβ variants and peptide mixtures were incubated at the concentration and buffer conditions used to study vasoactivity and secondary structure. Under these conditions and at the time the vasoconstriction and CD assays were carried out (2 h) there was no significant peptide aggregation in any of the samples, as assessed by centrifugation and peptide quantitation in the supernatant. These results suggest that differences in vasoactivity are not due to distinct degrees of peptide polymerization. We cannot rule out the possibility that slight differences in peptide oligomerization (such as dimers, tetramers, octamers, etc.) could contribute to the observed vasoactivity and it is conceivable that heterodimeric or heteropolymeric forms of the peptides, specifically those created between wild type $A\beta_{1-40}$ or $A\beta_{1-42}$ and $A\beta(22Q)_{1-40}$, are more vasoactive than homodimers or homopolymers.

The finding that peptides containing low or high levels of β-sheet conformation are less vasoactive than those containing intermediate amounts of β-sheet (Fig. 2B) leads us to propose the existence of a transitional form between random coil and β-pleated that is the vasoactive species of Aβ. To our knowledge, this is the first time that a biological activity has been associated with an Aβ conformational intermediate. In addition, the results showing that the mixture between $A\beta_{1-40}$ and the less vasoactive $A\beta(22Q)_{1-40}$ or $A\beta_{1-42}$ produces a peptide which exhibits high vasoactivity and intermediate conformation, indicate that different Aβ peptides could interact among themselves and modify their properties.

It is possible that such interactions could modify the cytotoxic properties of these peptides in vascular cells. For example, freshly solubilized $A\beta_{1-42}$ and $A\beta(22Q)_{1-40}$ are more toxic to cerebrovascular smooth muscle cells than $A\beta_{1-40}$, but increasing the β-sheet content of $A\beta_{1-42}$ by preaggregation abolishes the toxic effects of $A\beta_{1-42}$ [16]. In human aortic endothelial cells, freshly solubilized $A\beta_{1-42}$ is again more toxic than $A\beta_{1-40}$, but while aging $A\beta_{1-40}$ increases the toxicity, there is no effect on $A\beta_{1-42}$ [5]. These data suggest that, as with vasoactivity, toxicity of Aβ to vascular cells might be modified by interaction between Aβ peptides of differing β-sheet content.

We have previously shown that the enhancement of vasoconstriction by Aβ peptides is associated with rapid degeneration of the vessel wall [7], and these effects of Aβ in rat aortae have also been demonstrated in bovine middle cerebral artery [17]. Both free radical and intracellular calcium levels have been demonstrated to modify the effects of Aβ [8], but the mechanism of enhanced vasoconstriction or the accompanying vessel damage in this experimental set-up is as yet unknown. We hypothesize that in-life contact of Aβ peptides in the vasoactive form with small cerebral vessels will increase their tendency to constrict. The resultant subclinical ischemia would be expected to up-regulate βAPP production in the vasculature with further increase in Aβ formation. Such a cycle of events would be expected to contribute to the destruction of cerebral vessels in both CAA and HCHWA-D. This hypothesis is supported by the findings indicating that vascular amyloid deposits in HCHWA-D consist of a mixture of wild type $A\beta_{1-40}$ (with small amounts of $A\beta_{1-42}$) and $A\beta(22Q)_{1-40}$ [18]. Similarly, as the mutations responsible for early onset familial Alzheimer's disease all appear to result in an increase in the production of the $A\beta_{1-42}$ fragment from βAPP [19] one might postulate that $A\beta_{1-40}/A\beta_{1-42}$ peptide mixtures are associated with the CAA observed in Alzheimer's disease. In any case, it seems clear that neither the normal soluble form of Aβ nor the fibrillar state are the most vasoactive, but rather that a conformational intermediate is the most vasoactive and thus potentially the most pathogenic species.

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