Amyloid β oligomers constrict human capillaries in Alzheimer's disease

via signalling to pericytes

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Vascular compromise occurs early in Alzheimer's disease (AD) and other dementias¹⁻³. Amyloid β ($A\beta$) reduces cerebral blood flow⁴⁻⁶ and, as most of the cerebral vasculature resistance is in capillaries⁷, $A\beta$ might mainly act on contractile pericytes on capillary walls⁸⁻¹⁰. Employing human tissue to establish disease-relevance, and rodent experiments to define mechanism, we now show that $A\beta$ constricts brain capillaries at pericyte locations in human subjects with cognitive decline. Applying soluble $A\beta_{1-42}$ oligomers to live human cortical tissue constricted capillaries. Using rat cortical slices, this was shown to reflect $A\beta$ evoking capillary pericyte contraction, with an EC_{50} of 4.7 nM, via the generation of reactive oxygen species and activation of endothelin ET-A receptors. In freshly-fixed diagnostic biopsies from human patients investigated for cognitive decline, mean capillary diameters were less in subjects showing $A\beta$ deposition than in subjects without $A\beta$ deposition. For patients with $A\beta$ deposition, the capillary diameter was 31% less at pericyte somata than away from somata, predicting a halving of blood flow. Constriction of capillaries by $A\beta$ will contribute to the energy lack¹⁻³ occurring in AD, which promotes further $A\beta$ generation^{11,12}. This mechanism reconciles the amyloid hypothesis¹³⁻¹⁵ with the earliest events in AD being vascular¹.

Around the time that the amyloid hypothesis for Alzheimer's Disease (AD) was published¹³⁻¹⁵, it was reported that capillaries in the brains of AD patients showed an abnormal focally-constricted morphology^{16,17} similar to that produced by pericyte contraction^{8,9}. Although much succeeding work focused on $A\beta$ - and tau-evoked damage to neurons, increasing evidence suggests a role for vascular disturbance in the onset of AD: an idea formalised in 1993^{18,19}. This concept is supported by observations of reduced cerebral blood flow early in the development of the disease¹, and by the fact that reduced cerebral blood flow increases $A\beta$ production^{11,12}.

Investigations of the vascular effects of $A\beta$ and AD have focused on arteries and arterioles^{5,6,20}, but in the CNS the majority of the vascular resistance is located in capillaries⁷.

A subset of pericytes on capillary walls is contractile and can alter cerebral blood flow by adjusting their contractile tone^{8,9}, and in a rodent model of AD there are disturbances of unknown origin in the control of capillary blood flow¹⁰. We therefore investigated how pericytes were affected by A β ₁₋₄₂ oligomers, the molecular species believed to be responsible for A β 's toxic effects in AD^{21,22}. To maximise the relevance to human disease, unlike most AD-related studies on rodents, we used living human brain slices derived from neurosurgically-resected brain tissue (removed to access tumours) to study acute responses to A β , and rapidly-fixed human brain biopsy tissue (with or without A β deposition) to assess pericyte responses to long-term accumulation of A β in AD. The mechanisms of the effects seen in human tissue were defined in rodent brain slices.

Living human brain cortex slices were obtained from tissue removed during neurosurgical operations to access tumours (see Online Methods) and either fixed for immunohistochemistry or imaged live to study pericyte properties. Labelling the basement membrane with fluorescently-tagged isolectin B₄, or immunolabelling for the pericyte marker platelet-derived growth factor receptor (PDGFR β), revealed pericyte morphology. Pericytes were observed with a classical “bump-on-a-log” morphology on the straight parts of capillaries (Fig. 1a), or at their branch points, with processes extending along and around the capillaries (Fig. 1b). The mean distance between pericytes was 65.3 $\pm$ 0.4 μ m (for 94 pericytes imaged in tissue from 2 patients), 30% larger than in rodents⁹. As for arteriole smooth muscle (Fig. 1c), the processes of 36% of pericytes labelled for α smooth muscle actin (Fig. 1d), providing a mechanistic substrate for the A β -evoked contraction described below. Superfused noradrenaline constricted and glutamate dilated the capillaries at pericyte locations (Fig. 1e, f), as previously reported for rodent capillaries⁸, confirming that the surgery-derived human tissue had functioning contractile pericytes.

A β was oligomerised (see Online Methods), and silver staining of SDS-PAGE gels was used to assess the degree of aggregation of the A β isoforms. The predominant species produced (other than monomers) for A β ₁₋₄₂ and A β ₁₋₄₀ had a molecular weight of 4 to 5 times

that of monomers, whereas scrambled A β_{1-42} formed mainly monomers (Fig. 1g). Applying soluble A β_{1-42} (oligomeric + monomeric, 72 nM) to human brain slices evoked a slowly developing constriction of capillaries that reduced their diameter by ~25% ($p=0.01$) after 40 mins (Fig. 1h). For a fixed pressure across the capillary bed, a focal constriction of this magnitude at pericyte somata is predicted to reduce cerebral blood flow by 45% if it occurred throughout the capillary network (see Online Methods).

As the limited availability of live human tissue precludes detailed analysis of the mechanism underlying the A β -evoked constriction, we carried out experiments on cortical slices from 21-day-old rats to investigate this. As for human capillaries, A β_{1-42} and also A β_{1-40} evoked a constriction of rat capillaries near pericyte locations that was visible using either bright field illumination or 2-photon fluorescence imaging of isolectin B₄ (Fig. 2a-c). The time course of the A β -evoked constriction (Fig. 2c) was similar to that in human cortex (Fig. 1h), reaching ~16% after 1 hour ($p=0.006$ for A β_{1-42} and 0.048 for A β_{1-40}). Capillaries monitored for an hour without applying A β , or those to which a version of A β_{1-42} with a scrambled sequence was applied (prepared as for the A β oligomers), showed no significant diameter change (Fig. 2c). Scrambled A β_{1-42} does not form oligomers (Fig. 1g), unlike A β_{1-42} and A β_{1-40} , which may indicate that oligomer formation is obligatory for an effect on pericytes. The pericyte-mediated constriction evoked by A β_{1-42} showed a Michaelis-Menten dependence on A β concentration, with an apparent EC₅₀ of 4.7 nM (Fig. 2d).

The A β_{1-42} -evoked capillary constriction in rat cortical slices was blocked by the endothelin-1 (ET-1) type A receptor blocker BQ123 (1 μ M, $p=0.008$), or by application of superoxide dismutase 1 (SOD1, 150 units/ml, $p=3.7\times 10^{-6}$) which scavenges reactive superoxide generated when A β activates NADPH oxidase in immune cells (Fig. 2e), as previously reported for the effect of A β on arteries and isolated penetrating arterioles^{5,6,20}. Neither of these agents alone affected capillary diameter (BQ123: $-0.7\pm 5.2\%$ dilation, $n=13$; SOD1: $3.4\pm 5.8\%$ dilation, $n=9$). Unlike for A β_{1-40} effects on blood flow *in vivo*⁵, blocking

binding of A β to the receptor for advanced glycation endproducts (RAGE) using FPS-ZM1 (1 μ M) had no effect on the pericyte constriction ($p=0.34$, Fig. 2e), reflecting the fact that we applied A β in the extracellular fluid: RAGE is only needed to transport A β into the brain from the blood⁵. To confirm that pericytes constrict in response to activation of ET-1 type A receptors, we applied ET-1 (10 nM) either alone or with a blocker of its type A or type B receptors. ET-1 evoked a strong pericyte-mediated constriction of capillaries ($p=2\times 10^{-12}$), which was blocked by the type A receptor blocker BQ123 (1 μ M, $p=2.6\times 10^{-11}$), but not by the type B receptor blocker BQ788 (1 μ M, $p=0.91$, Fig. 2f). ET-1 still evoked a constriction in the presence of SOD1 ($p=1.3\times 10^{-8}$, Fig. 2g) implying that ET-1 acts downstream of superoxide, while generating reactive oxygen species using H₂O₂ evoked a constriction ($p=1.1\times 10^{-5}$) that was reduced by BQ123 ($p=0.009$, Fig. 2h) suggesting that H₂O₂ constricts pericytes via endothelin receptor activation. These data establish A β_{1-42} -evoked generation of reactive oxygen species, presumably by resident microglia²³ or perivascular macrophages²⁴, as being upstream of the elevated level^{25,26} (or potentiated effect²⁷) of endothelin which makes pericytes constrict capillaries.

In profound ischaemia, pericyte-evoked constriction of capillaries is followed by the pericytes dying in rigor (probably⁹ via an excessive rise of [Ca²⁺]_i), thus maintaining a decreased capillary diameter and a long-lasting decrease of blood flow⁹, and pericytes also die after accumulating A β in AD²⁸. We assessed whether exposure to 1.4 μ M soluble A β_{1-42} or 100 nM ET-1 for 3 hours had a similar effect on pericyte health, by applying propidium iodide to label cells with membranes that had become non-specifically permeable⁹. These procedures did not significantly increase pericyte death on this time scale (Fig. 2i, $p=0.85$ for A β_{1-42} and 0.59 for ET-1).

Since acute exposure to A β cannot mimic the slow increase which occurs over decades in human AD patients, we studied rapidly-fixed brain cortical biopsy tissue from patients being investigated for cognitive decline of unknown cause. Tissue sections were labelled with antibodies to A β (recognising residues 8-17 of A β), PDGFR β and

phosphorylated tau. Of 13 patients (for demographics, see Online Methods), 7 turned out to have A β deposition while 6 did not. Specimen images of their vessels and A β labelling are shown in Fig. 3a, b. Pericytes were readily identifiable from their PDGFR β labelling. Averaging over 120-140 adjacent fields of view (400 μ m square in size, randomly placed on each section as a 5x4 grid of squares) in tissue from the two types of patient, with the analyst blinded to the occurrence of A β deposition (viewing only the PDGFR β image channel), we found that the mean capillary diameter was reduced by 8.1% ($p=0.0007$) in the patients with A β deposition (5121 diameters measured) compared to those without A β deposition (3921 diameters measured, Fig. 3c).

To assess whether this diameter reduction was a non-specific effect of AD, or was pericyte-related, we plotted the capillary diameter measurements as a function of the distance from the nearest PDGFR β -labelled pericyte soma (see Online Methods). In patients with no detectable A β deposition the capillary diameter increased at locations near pericyte somata compared to at locations far from the soma (~25% larger, slope of line is significantly less than zero, $p=3.7\times 10^{-7}$ for 813 data points from 6 such patients, Fig 3d). A similar increase in capillary diameter near somata has previously been found in rodent brain capillaries *in vivo*⁹, and attributed to the presence of the soma inducing more growth of the endothelial tube. In contrast, in patients with A β deposition, the capillary diameter was significantly reduced near the pericyte somata compared to locations distant from the somata (Fig. 3d; ~30% smaller, slope of line is significantly greater than zero, $p=1.6\times 10^{-20}$ for 1313 data points from 7 patients), as expected if pericytes cause the capillary constriction by contracting their circumferential processes which are mainly located near their somata. This constriction is predicted to reduce flow by ~50% compared to if there were no constriction (see Online Methods).

The subjects were classified by neuropathologists assessing the A β -labelled biopsies as having “no A β deposition”, “moderate A β deposition” or “severe A β deposition” in the parenchyma (as diffuse deposits and/or as plaques with central amyloid cores, Fig. 3e). The

mean slope from graphs like those in Fig. 3d, for 6 patients with no A β deposition, 3 patients with moderate deposition and 4 patients with severe A β deposition, showed a progressive change from negative (implying a larger capillary diameter at the soma) to positive (implying a smaller diameter at the soma) as the severity of the A β deposition increased (Fig. 3f, $p=0.003$ compared with a relationship with zero slope), supporting further the idea that A β is the cause of the capillary constriction. Plotting a similar graph for the slope against the neuropathologist-assessed degree of cerebral amyloid angiopathy or phosphorylated tau labelling, gave a broadly similar trend with disease severity, but with a somewhat less significant p value (0.013 and 0.007 respectively).

To quantify A β level more rigorously, we measured light absorption by the peroxidase product generated by the A β antibody, in the region where the vessel diameters were measured in each biopsy (see Online Methods; although this measure of A β may largely reflect the presence of plaques, it is likely that the soluble A β concentration correlates with plaque load). Plotting the slopes of graphs like those in Fig. 3d, for each biopsy, as a function of the amount of A β deposition, again showed a monotonic progression from a negative slope to a positive slope as A β deposition increased, but with the change of slope occurring more strongly at low levels of A β deposition (Fig. 3g). Similarly, plotting the value of the capillary diameter at the pericyte soma for each biopsy (extrapolated from the straight line fit) as a function of A β deposition showed that the diameter was reduced more strongly by low levels of A β , with less further constriction as deposition increased (Fig. 3h).

Our data demonstrate that, at low nanomolar concentrations, soluble A β_{1-42} oligomers evoke a constriction of human cortical capillaries, mediated by pericytes. Capillaries are the site in the cortical vasculature where most of the resistance to flow is located⁷, and so are the site where diameter changes will reduce blood flow most (although arteries²⁰ and arterioles⁶ are also reported to be constricted by A β). In rodents we show that this constriction is the result of reactive oxygen species generation activating endothelin signalling via ET_A receptors, which makes pericytes constrict the capillaries. The EC₅₀ for

A β 's action, 4.7 nM, is comparable to the concentration of soluble A β found in the brain (6 nM, from the TBS fraction in Table 1 of ref. 29) and two factors indicate that the effects seen are pathologically relevant. First, analysing the diameter of capillaries in human patients with cognitive decline, who either showed or lacked deposition of A β , shows that Alzheimer's pathology leads to capillary constriction specifically at pericytes, with a magnitude that is estimated to reduce blood flow by a factor of at least 2. Second, the magnitude of the capillary constriction in dementia patients increases with the severity of A β deposition.

Both the reduction of basal blood flow produced by A β , and a reduction in the blood flow increase normally produced by neuronal activity³⁰ (which may also reflect the action of A β on pericytes⁹), will decrease the energy supply to the brain. This in turn increases A β production by upregulating β -amyloid converting enzyme (β -secretase 1, BACE1)^{11,12}. Consequently, the regulation of pericyte-mediated capillary constriction by A β may act as an amplifying mechanism in a positive feedback loop (Fig. 4), increasing the level of A β and tau aggregation which ultimately lead to the loss of synapses and neurons.

These data suggest several potential therapeutic approaches for early AD, based on the mechanisms generating pericyte constriction. A β -evoked generation of reactive oxygen species (ROS) might be targeted, but this would require resolving whether these are generated by resident microglia²³ or perivascular macrophages²⁴, and ROS may play important signalling roles in pathways other than the one controlling endothelin effects. A more promising approach, therefore, might be to try to reduce endothelin release (which is presumably from microglia or endothelial cells: the brain cells expressing endothelin strongly) or the effects of endothelin specifically on its type A receptors located on CNS pericytes. This could be achieved, for example, by generating an endothelin receptor blocker which also binds to another target protein on pericytes, such as the proteoglycan NG2. This concept raises the question of whether A β also evokes constriction of capillaries in other vascular beds by acting through pericytes, in which case drugs might be usefully targeted at all pericytes, and not just those in the brain.

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Online Methods

Human brain slices

The work received ethical approval from the National Health Service (REC number 15/NW/0568) and all patients gave informed consent. During neurosurgical operations for tumour treatment, apparently normal cortical tissue that was removed (to gain access to the tumour), which would otherwise have been discarded, was placed in ice cold brain slicing solution containing (mM) 93 N-methyl-D-glucamine (NMDG) chloride, 2.5 KCl, 30 NaHCO₃, 10 MgCl₂, 1.2 NaH₂PO₄, 25 glucose, 0.5 CaCl₂, 20 HEPES, 5 Na ascorbate, 3 Na pyruvate, 1 kynurenic acid (to block glutamate receptors, to prevent excitotoxic damage to neurons), oxygenated by gassing with 95% O₂/5% CO₂, and transported in less than 15 mins to the laboratory. Tissue was cut into 200 µm sections and the slices were incubated at 34°C in the same solution for 10 mins, and then incubated at room temperature until used in experiments in a similar solution³¹ with the NMDG-Cl, MgCl₂ and CaCl₂ replaced by (mM) 92 NaCl, 1 MgCl₂ and 2 CaCl₂. Each patient's tissue typically generated ~2 brain slices. When sufficient tissue was present, histological examination of the slices using haematoxylin and eosin by neuropathologists was used to assess tumour infiltration into the nominally normal tissue. This revealed that while some slices showed no infiltration by the tumour, others did. Aβ was only applied to slices that showed no tumour infiltration. Pericyte responses to noradrenaline and glutamate as documented in Fig. 1 were observed whether or not there was tumour infiltration.

Rodent brain slices

Experiments used P21 Sprague-Dawley rats of either sex. All animal procedures were carried out in accordance with UK regulations. Cerebral cortical slices (300 µm thick) were prepared⁸ and stored as for human slices.

Extracellular solution

Brain slices were superfused at ~4 ml/min with solution containing (mM) 124 NaCl, 2.5 KCl, 26 NaHCO₃, 1 MgCl₂, 1 NaH₂PO₄, 10 glucose, 2 CaCl₂. This solution was gassed

with 20% O₂/75% N₂/5% CO₂, which produces a physiological level of oxygen in the slice near the capillaries being imaged⁹.

Imaging capillaries

Healthy capillaries (<10 μm in diameter, mean diameter 5.61±0.03 μm (n=299) in rat, and 5.08±0.33 μm (n=12) in human, with no rings of arteriolar smooth muscle around them) were selected as previously described³¹, and regions of them were imaged which were in focus in a single image plane over at least 30 μm along the length of a capillary and exhibited a candidate pericyte with a bump-on-a-log morphology (Figs. 1e, 2a). A CCD camera was then used to capture images 100 μm square during superfusion of drugs. Capillary diameter was measured from the resulting movies, by an analyst blinded to the time and identity of drug application, by placing a line across the lumen on magnified images. In some experiments pericytes were identified prior to imaging by incubating slices for 30 min in 10 μg/ml isolectin B₄ conjugated to Alexa 488, which binds to α-D-galactose residues in the basement membrane secreted by pericytes and endothelial cells, and outlines pericytes³¹. This allowed 2-photon imaging (using a Zeiss LSM710 microscope, excitation wavelength 800 nm) of the endothelial tube and the pericytes on it (Fig. 2b).

Assessing pericyte death

This was carried out as previously described⁹. Briefly, brain slices (250 μm thick) were incubated in a multi-well plate, with oxygen blown gently at the surface, in aCSF, or aCSF with oligomerised Aβ₁₋₄₂ or ET-1 added. All extracellular solutions contained isolectin B₄ to label the basement membrane, and hence pericytes which are enveloped by this (Fig. 1b), and 7.5 μM propidium iodide to label cells with membranes that had become non-specifically permeable⁹. After 3 hours incubation, slices were fixed in 4% paraformaldehyde, and imaged on a confocal microscope. To avoid counting cells killed by the slicing procedure, quantification of the percentage of pericytes that were dead excluded cells within 20 μm of the slice surface.

Oligomerising A β and assessing the form and concentration of A β applied

The method employed to generate oligomeric A β preparations was modified from that previously described³². Synthetic A β_{1-42} (Bachem H-1368.1000), A β_{1-40} (Bachem H-1194.1000) and scrambled A β_{1-42} (Bachem H-7406.1000) were suspended in 1,1,1,3,3,3 hexafluoro-2-propanol (HFIP; 52527, Sigma) at 1 mM, vortexed to obtain a homogenous solution, and aliquoted to microcentrifuge tubes. The HFIP was removed by overnight evaporation and the A β was completely lyophilised via a Speed-Vac. The A β peptide films were stored desiccated at -20°C until further processed. The peptide films were then resuspended at a nominal 5 mM in DMSO, bath-sonicated for 10 min and vortexed for 30 sec. To form A β oligomers, this solution was diluted to a nominal 100 μ M with phosphate-buffered saline, vortexed for 15-30 sec and incubated at 4°C for 24 h. Immediately before use, the oligomeric preparations were centrifuged at 14,000 g for 10 min at 4°C (to remove any fibrils that might be present) and the supernatants were further diluted to the final experimental concentrations (quantified below) with extracellular solution.

Quantification of A β peptide concentration was performed using a Pierce BCA protein assay kit (Thermoscientific 23227), calibrated against a known concentration of bovine serum albumin, taking into account the different chromophoric development of albumin and A β peptides by multiplying by a factor of 1.51^{33,34}. This showed that the amount of the molecule remaining as soluble monomers and oligomers was 28.7 $\pm$ 2.9% (n=4) of the total concentration added for A β_{1-42} , 39.9 $\pm$ 1.5% (n=3) for A β_{1-40} , and 43.6 $\pm$ 2.3% for scrambled A β_{1-42} . Concentrations stated in the text have been corrected for these factors.

The A β oligomeric preparations were analysed via SDS-PAGE using 10-20% tris-glycine gels (EC61352BOX, Invitrogen). Samples of 50 μ g A β peptides were added to tris-glycine SDS Sample Buffer (LC2676, Invitrogen). Equal volumes of each sample (10 μ l) were loaded onto gels along with SeeBluePlus2 (Invitrogen) pre-stained molecular weight markers, and electrophoretically separated at 100 V. Gels were stained for total protein using a SilverXpress Silver Staining kit (LC6100, Invitrogen) according to the manufacturer's

protocol. A β ₁₋₄₂ and A β ₁₋₄₀ formed monomers and oligomers, while scrambled A β ₁₋₄₂ formed only monomers (Fig. 1g).

Human biopsy data

Diagnostic brain biopsies, comprising cortex and subcortical white matter, were performed as part of routine clinical investigation at the National Hospital for Neurology and Neurosurgery, Queen Square, London, to exclude treatable causes of neurological symptoms the patients had presented with. All patients gave informed consent for the biopsy. The use of human tissue samples was licensed by the National Research Ethical Service, UK (University College London Hospitals NRES license for using human tissue samples, project ref 08/0077). The storage of human tissue was licensed by the Human Tissue Authority, UK (License #12054).

Biopsies (volume typically 1 cm³) were all from the right frontal lobe. The biopsies were fixed in 10% buffered formalin less than 30 mins after the resection, for a minimum of 12 hours. The formalin fixed tissue was dehydrated through graded alcohols and embedded in paraffin wax, from which 4 μ m thick sections were cut for routine haematoxylin and eosin staining and a panel of immunohistochemical stains. As part of the diagnostic work up, the sections were immunostained for A β with immunoperoxidase-labelled antibody 6F3D (DAKO, 1:50), for phosphorylated tau with antibody AT8 (Innogenetics, 1:100), and for this study in addition with antibody against PDGFR β (RD systems, cat. no. MAB1263, 1:20) to label pericytes. This was performed on a Roche Ventana Discovery automated staining platform following the manufacturer's guidelines, using biotinylated secondary antibodies and streptavidin-conjugated horseradish peroxidase and diaminobenzidine as the chromogen. The extent of parenchymal A β deposition was assessed semi-quantitatively as absent, moderate or severe by a neuropathologist. In addition, to objectively quantify A β deposition, the images of the immunoperoxidase label for A β were imported into ImageJ, and split into red, green and blue channels. Then, the light intensity in the blue channel (which gave best distinction of the immunoperoxidase label from the background tissue

haemotoxylin labelling) was measured in the region of the biopsy where diameters were measured, normalised by the intensity in a region of the section showing no visible A β label and converted to a percentage of light absorbed by the A β . Normalising by the intensity in a (tissue-free) region without any tissue absorption gave values that were $5.8 \pm 0.5\%$ larger, which did not materially change the form of the graphs.

The mean age of patients without A β deposition was 50.5 ± 5.5 (n=6, 4 women and 2 men), and of those with A β deposition was 62.1 ± 4.2 (n=7, 4 women and 3 men, not significantly different, $p=0.11$). Regressing mean capillary diameter against age from all patients, or from the patients lacking A β deposition, showed that there was no significant dependence on age ($p=0.5$ and $p=0.82$ respectively).

Images were analysed to assess capillary diameter with the analyst blind to the level of A β and tau deposits (viewing only the PDGFR β channel). A standard 5x4 grid of 20 squares (each with sides 400 μ m long) was superimposed on each image, and all capillaries with clearly demarcated endothelial walls visible in each square had their diameter measured. The image squares were treated as the experimental unit for statistical analysis. Analysis of the diameter as a function of distance from the nearest visible pericyte employed a subset of all the measured diameters, because often no pericyte was visible on some short capillary segments.

Immunohistochemistry of non-biopsy tissue

Human and rat brain slices were fixed in 4% paraformaldehyde for 1 hour, washed 3 times in phosphate-buffered saline (PBS), then blocked in goat serum. Primary antibodies for PDGFR β (Santa Cruz, cat. no. sc432, 1:200) or α -SMA (Santa Cruz, cat. no. CGA7, 1:200) were applied overnight, followed (after washing in PBS) by application overnight of Alexa Fluor 647 conjugated secondary antibodies (ThermoFisher, cat. no. A-21245, 1:200). Slices were then washed once in PBS containing DAPI nuclear stain (1:50,000) for 10 mins and then washed again in PBS. After cover-slipping, slices were imaged on a Zeiss LSM700 confocal microscope.

Statistics

Data normality was assessed with Shapiro-Wilk tests. Comparisons of normally distributed data were made using 2-tailed Student's t-tests. Equality of variance was assessed with an F test, and heteroscedastic t-tests were used if needed. Data that were not normally distributed were analysed with Mann-Whitney tests. P values were corrected for multiple comparisons using a procedure equivalent to the Holm-Bonferroni method (for N comparisons, the most significant p value is multiplied by N, the 2nd most significant by N-1, the 3rd most significant by N-2, etc.; corrected p values are significant if they are less than 0.05). Assessment of whether the slope of linear regressions differed significantly from zero was obtained using the t-statistic for the slope. P values comparing vessel diameters in the absence and presence of drugs were calculated for the last data point in each graph shown, or for an exposure time of 45-60 mins if no graph is shown. An estimate of the sample size needed for a typical experiment is as follows: For a control response of 100%, a response standard deviation of 10%, a response in a drug of 70% (30% inhibition), a power of 80% and $p < 0.05$, <6 vessels are needed in each of the control and drug groups (www.biomath.info/power/ttest.htm). The exact numbers depend on the drug effect size and standard error of the data.

Calculation of effect of vessel constriction on flow

We assume that pericytes are regularly spaced on capillaries at an interval of $2L$. For flow governed by Poiseuille's law, the resistance of a segment of capillary of length L (from a pericyte soma to midway between two pericytes) and radius r_1 is given by

$$k.L/r_1^4$$

where k is a constant. If $A\beta$ -induced pericyte contraction reduces the capillary diameter from a value of r_1 at the midpoint between pericytes to r_2 near the pericyte soma (see Fig. 3a, b, d), then if this reduction is linear with distance the resistance of the capillary segment from the soma to the midpoint is given by

$$k.L.(r_1^2 + r_1.r_2 + r_2^2)/(3.r_1^3.r_2^3)$$

so the factor by which the resistance is altered (compared to that with a uniform diameter r_1) is

$$[1 + (r_1/r_2) + (r_1/r_2)^2] \cdot (r_1/r_2)/3 \quad (1)$$

This was used to calculate the predicted flow reduction to be produced by the 30% pericyte constriction reported at pericyte somata in Fig. 3d and the main text. In reality the flow reduction will be greater because the diameter at the pericyte soma is actually larger than at the midpoint between pericytes in control conditions (Fig. 3d), and because Poiseuille's law does not apply for small capillary diameters for which the effective blood viscosity increases as the diameter decreases below $10 \mu\text{m}$ ³⁵.

Data availability

The data generated in the current study are available from the corresponding author on reasonable request

Figure Legends

Figure 1. Oligomeric A β acts on pericytes to constrict capillaries in human brain slices.

(a) Isolectin B₄ labelled capillary in a human cortical slice, with two pericyte somata (arrowheads) outlined by their basement membrane. (b) Pericyte labelled with antibody to PDGFR β . (c-d) Arteriole (c) and pericyte (d) labelled with isolectin B₄ and antibody to α smooth muscle actin (SMA). (e) Images of a capillary (dashed lines indicate wall) and pericyte soma (arrowhead) in a live brain slice before drug application (*before*), in the presence of 2 μ M superfused noradrenaline (+NA), with 2 μ M NA and 500 μ M glutamate superfused (+NA +Glu), and after stopping drug superfusion (*washout*). Graph shows time course of capillary diameter at right red line throughout the experiment. (f) Mean glutamate-evoked dilation and noradrenaline-evoked constriction in experiments as in (e) (change in diameter quantified relative to that before application of each drug; relative to the pre-noradrenaline diameter the glutamate-evoked dilation was $26.8 \pm 7.7\%$). (g) Silver staining of an SDS-PAGE gel for A β solutions prepared as in the Online Methods. (h) Images of a capillary before and after superfusion of 72 nM A β_{1-42} , showing a region (red line) being constricted by pericytes and one region (yellow line) that is not constricted. Graph shows mean diameter change at 4 pericyte locations from 3 slices treated with A β and 3 pericyte locations from 3 slices treated with aCSF (significantly reduced at 40 mins in A β , $p=0.01$).

Figure 2. A β acts via reactive oxygen species and endothelin type A receptors.

(a-b) Bright field images (a) and 2-photon images of IB₄ fluorescence (b) of capillaries in rat cortical slices in normal aCSF and after superfusion with 72 nM A β_{1-42} , showing capillary constriction near pericytes. (c) Mean time course of normalised capillary diameter during superfusion with aCSF ($n=51$ vessels), scrambled A β_{1-42} (109 nM, $n=32$), A β_{1-42} (72 nM, $n=20$) or A β_{1-40} (100 nM, $n=6$). (d) Constriction produced after 1 hour by different concentrations of A β_{1-42} ($n=51, 11, 10, 19$ and 20 for $0, 2.9, 14, 57$ and 72 nM respectively). Curve through the points is a Michaelis-Menten relation with a best-fit K_m of 4.7 nM and a

maximum constriction of 16.1%. **(e)** Mean time course of normalised capillary diameter during superfusion with 57 nM A β ₁₋₄₂ alone (n=19), or in the presence of superoxide dismutase 1 (SOD1, 150 units/ml, n=19), the ET-A receptor blocker BQ123 (1 μ M, n=14), or the RAGE blocker FPS-ZM1 (1 μ M, n=8). **(f)** Mean time course of normalised capillary diameter during superfusion with aCSF (n=10), ET1 alone (10 nM, n=10), or ET1 in the presence of the ET-A receptor blocker BQ-123 (1 nM, n=10) or the ET-B receptor blocker BQ-788 (1 μ M, n=12). **(g)** SOD1 (150 U/ml, n=8) does not block the constriction evoked by endothelin (ET1, 5 nM, n=12). **(h)** The reactive oxygen species generator H₂O₂ (1 mM, n=9) evokes capillary constriction ($p=1.1 \times 10^{-5}$ at 20 mins) which is attenuated by the ET-A receptor blocker BQ123 (1 μ M, n=11, $p=0.009$). **(i)** Incubating rat brain slices in solution containing A β ₁₋₄₂ oligomers (1.4 μ M, n=28) or ET1 (100 nM, n=30 slices) for 3 hours does not increase pericyte death over that seen when incubating in aCSF (n=27).

Figure 3. Pericyte-mediated capillary constriction occurs in humans with A β deposits.

(a-b) Sample images of human cortical biopsies, labelled for PDGFR β (brown in top panels) to show pericytes (arrows), from patients lacking **(a)** or exhibiting **(b)** A β deposits (brown in bottom panels, haematoxylin counterstain blue). Red lines indicate capillary diameter. **(c)** Mean diameter of capillaries in patients lacking (3921 diameters measured) or exhibiting (5121 diameters measured) A β deposits (number of images analysed shown on bars). **(d)** Dependence of capillary diameter on distance from a visible pericyte soma (in 5 μ m bins, from 0-5, 5-10, 10-15 and 15-20 μ m) for patients lacking or exhibiting A β deposits (moderate and severe A β deposition pooled together). P values assess whether slope of regression line is significantly different from zero. **(e)** Examples of A β labelling assessed by the neuropathologist as absent, moderate or severe. **(f)** Slope of regression lines as in d plotted as a function of neuropathologist-rated parenchymal A β load. **(g)** Slope of regression lines as in d plotted as a function of severity of A β deposition measured optically, with subjects grouped by colour (defined in f) as classified by neuropathologist. **(h)** Dependence of extrapolated diameter at soma (as in d) on severity of A β deposition measured optically, with

subject points coloured as classified by neuropathologist (colours defined in f). Lines through data in f-h are to show the trends in the data.

Figure 4. A β effects on capillaries may amplify the onset of AD.

Amyloid β oligomers activate NADPH oxidase in immune cells^{23,24} to generate reactive oxidative species (ROS). These in turn release, or potentiate the constricting effects of, endothelin which acts via ET_A receptors on pericytes on capillaries - the locus⁷ of the largest component of vascular resistance within the brain parenchyma. Capillary constriction decreases cerebral blood flow and hence the supply of oxygen and glucose to the brain, which increases the production of A β , in part by upregulating^{11,12} expression of β -amyloid converting enzyme (β -secretase 1, BACE1), thus forming an amplifying positive feedback loop. Either directly, or via tau production, or via the decrease in oxygen and glucose supply, a rise in A β concentration leads to the loss of synapses and neurons. Potential sites for therapeutic intervention are highlighted at the stages of ROS (SOD1) and endothelin receptors (BQ123).

Fig. 1

Fig. 2

Fig. 3

Fig. 4