

Age-dependent cerebrovascular dysfunction in a transgenic mouse model of cerebral amyloid angiopathy

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The Tg2576 transgenic mouse model of human cerebral amyloid angiopathy is characterized by age-dependent cerebrovascular deposition of amyloid- β (A β) starting from 9 months of age and progressively worsening to involve most pial arterioles by 18 months; soluble A β levels are elevated long before vascular deposition takes place in this model. It has been suggested that elevated soluble A β levels alone are sufficient to impair cerebral blood flow (CBF) regulation thereby contributing to the early progression of Alzheimer's disease. Using laser speckle flowmetry through an intact skull, we studied the impact of elevated soluble A β levels and vascular A β deposition on a wide range of CBF responses to evaluate vasodilation and vasoconstriction in young or aged Tg2576 mice. Nineteen-month-old Tg2576 with severe vascular A β deposits showed an attenuated hyperaemic response during hypercapnia and whisker stimulation compared to wild-type littermates. The anticipated increase in CBF due to isoflurane anaesthesia was also suppressed, as were the typical hypoperfusion responses during cortical spreading depression and α -chloralose anaesthesia. The responses of 8-month-old Tg2576 with elevated soluble A β levels, but without vascular A β deposition, did not differ from age-matched controls. In conclusion, our data suggest that vascular A β deposition is associated with impaired vasodilator as well as vasoconstrictor responses to a wide range of stimuli. These responses do not differ from controls when studied non-invasively prior to vascular A β deposition, thus challenging the view that elevated soluble A β levels are sufficient to cause cerebrovascular dysfunction.

Keywords: cerebral blood flow; laser speckle flowmetry; hypercapnia; whisker stimulation; spreading depression

Abbreviations: CAA = cerebral amyloid angiopathy; CSD = cortical spreading depression; LSF = laser speckle flowmetry

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Introduction

Amyloid- β (A β) peptides derived from proteolytic processing of the β -amyloid precursor protein (APP) characteristically accumulate as cerebral parenchymal plaques in Alzheimer's disease (AD). Deposition of A β peptide in cerebral vessel walls, termed cerebral amyloid angiopathy (CAA), is found in the majority of AD brains. It is well established that CAA leads to ischaemic infarcts as well as lobar intracerebral haemorrhages, and thus may exacerbate the cognitive decline in AD (Vonsattel *et al.*, 1991; Greenberg *et al.*, 1995; Olichney *et al.*, 1995). In support of this, recent studies in transgenic mouse models of AD

and CAA demonstrated an association between elevated soluble A β levels and impaired cerebrovascular function (Iadecola, 2004). Importantly, this association was apparent prior to vascular A β deposition, raising the possibility that elevated soluble A β levels in AD brain may adversely impact progression of the disease via hemodynamic mechanisms. However, there is as yet no clinical evidence to support cerebrovascular dysfunction in young asymptomatic individuals destined to develop AD or CAA. Furthermore, evidence is also lacking for a hemodynamic mechanism hastening the cognitive decline in AD, except when overt ischaemic or haemorrhagic strokes introduce

Table 1 Arterial pressure and blood gases in Tg2576 mice

Strain	Age (mo)	BP (n)		pH	pO ₂	pCO ₂	
		α -Chloralose	Isoflurane			Baseline	5% CO ₂
Wild type	3	78 ± 6 (9)	81 ± 2 (6)	7.34 ± 0.04	131 ± 25	39 ± 5	62 ± 8
Wild type	8	83 ± 9 (5)	71 ± 9* (5)	7.32 ± 0.07	126 ± 24	39 ± 6	64 ± 7
Tg2576	8	90 ± 7 (7)	94 ± 6 (4)	7.30 ± 0.04	128 ± 14	40 ± 6	63 ± 4
Wild type	19	87 ± 5 (16)	91 ± 5 (8)	7.34 ± 0.03	139 ± 18	37 ± 3	58 ± 6
Tg2576	19	86 ± 4 (8)	93 ± 13 (5)	7.31 ± 0.04	131 ± 21	38 ± 5	63 ± 11

Note: Values are mean ± SD. BP (mean arterial blood pressure), pH, pO₂ and baseline pCO₂ (mmHg) are the average from all experimental paradigms, whereas pCO₂ during 5% CO₂ inhalation is the average during hypercapnia only. Numbers of animals (n) indicate the total number used in all experimental paradigms. *P < 0.05 versus α -chloralose.

additional neuropathological lesions (Snowdon *et al.*, 1997; Pfeifer *et al.*, 2002).

We, therefore, tested whether the presence of vascular amyloid deposits impact cerebrovascular responses in transgenic mice (Tg2576) overexpressing a mutant human APP associated with early onset familial AD and CAA (Hsiao *et al.*, 1996). In these experiments, blood flow served as a surrogate measure for the ability of vessels to dilate or constrict under diverse stimuli. In Tg2576 mice, brain A β levels are significantly elevated even at 2–3 months of age and levels rapidly increase starting around 7 months; parenchymal and vascular A β deposition appear after 9 months (Kawarabayashi *et al.*, 2001). With advanced disease (i.e. 19 months) severe A β deposition encase vascular smooth muscle cells and may cause cell loss (Christie *et al.*, 2001). Using a novel non-invasive optical imaging technique with high spatiotemporal resolution, we report that 8-month-old Tg2576 mice with elevated soluble A β but no vascular A β deposition show cerebrovascular responses that do not differ from wild-type mice when challenged with 5% inhaled CO₂, whisker stimulation or cortical spreading depression. In contrast, 19-month-old Tg2576 mice with widespread and severe vascular A β deposits exhibit profound impairment in the typical blood flow responses to the same experimental stimuli. The data we present in this manuscript do not support a vascular role for soluble A β in early AD pathogenesis.

Methods

Experimental animals

Heterozygous Tg2576 expressing Swedish double mutant APP (APP_{K670/671L}) driven by the hamster prion protein promoter (Hsiao *et al.*, 1996) on a mixed C57BL/6J-SJL/J background, and their wild-type littermates, were studied at 8 (28–29 g) or 19 months of age (33–39 g). In addition, 3-month-old wild-type mice were compared to 8- or 19-month-old wild-type mice to control for the effects of ageing. Only male mice were used in all groups. Eight-month-old Tg2576 mice show elevated A β levels but no vascular deposits, whereas 19-month-old mice accumulate severe parenchymal and vascular A β deposition with loss of smooth muscle cells, and thus represent an advanced stage of AD and CAA (Christie *et al.*, 2001; Domnitz *et al.*, 2005; Robbins *et al.*, 2006).

General surgical preparation and physiological monitoring

Mice were initially anaesthetized with isoflurane (2% induction, 1% maintenance, in 70% N₂O and 30% O₂) and intubated via a tracheostomy. Femoral artery was catheterized for blood pressure, heart rate and blood gas monitoring (ETH 400 transducer amplifier, CB Sciences, Milford, MA). Mice were paralysed (pancuronium bromide, 0.4 mg/kg/h, i.p.), mechanically ventilated (CWE, SAR-830, Ardmore, PA), placed in a stereotaxic frame (David Kopf, Tujunga, CA) and scalp and periosteum were retracted. At the end of surgical preparation, anaesthesia was switched from isoflurane to α -chloralose (50 mg/kg/h, i.v.) to preserve vascular responses (Ayata *et al.*, 2004a). Anaesthetic depth was adjusted to abolish cardiovascular reflexes to tail pinch. Rectal temperature was kept at 36.8–37.1°C using a thermostatically controlled heating mat (FHC, Brunswick, ME). Arterial blood gases and pH were measured immediately before a stimulus and at least once every hour, in 30 μ l blood samples (Corning 178 blood gas/pH analyzer, Ciba Corning Diagnostics, Medford, MA). These data (Table 1) were within the previously reported normal limits for mice and did not differ among groups (Dalkara *et al.*, 1995; Niwa *et al.*, 2001; Park *et al.*, 2004; Lacombe *et al.*, 2005). The data were continuously recorded using a data acquisition and analysis system (PowerLab, AD Instruments, Medford, MA).

Laser speckle flowmetry

Laser speckle flowmetry (LSF) (Dunn *et al.*, 2001) was used to study the spatiotemporal characteristics of CBF changes in mice as previously described in detail (Ayata *et al.*, 2004a). Briefly, the scalp was shaved, incised at midline and reflected laterally. A CCD camera (Cohu, San Diego, CA) was positioned above the head to image the entire right hemisphere (Fig. 1). A laser diode (780 nm) was used to illuminate the intact skull surface in a diffuse manner. The penetration depth of the laser is estimated to be ~500 μ m from the pial surface. Raw speckle images were used to compute speckle contrast, which is a measure of speckle visibility related to the velocity of the scattering particles, and therefore CBF. The speckle contrast is defined as the ratio of the standard deviation of pixel intensities to the mean pixel intensity in a small region of the image. Ten consecutive raw speckle images were acquired at 15 Hz (an image set), processed by computing the speckle contrast using a sliding grid of 7 \times 7 pixels and averaged to improve signal-to-noise ratio. Relative CBF images (percentage of baseline) were

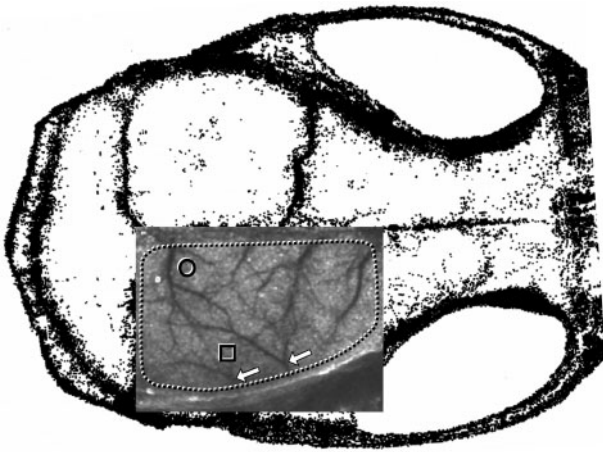


Fig. 1 The imaging field for laser speckle flowmetry. Speckle contrast image superimposed on mouse skull showing the position of imaging field (5.24×7 mm) in all experiments. Imaging was performed through intact skull. Surface vessels are seen as dark branching structures. Arteries and veins are identified based on their size and anatomical location. Arrows point to the middle cerebral artery branches. Circle and square show the ROIs used to quantify the CBF changes during CSD and whisker stimulation, respectively. Dotted line outlines the ROI used to quantify the CBF changes during hypercapnia.

calculated by computing the ratio of a baseline image of correlation time values (τ_c) to subsequent images.

Experimental paradigms

Hypercapnia

Hypercapnia was induced by 5% CO_2 inhalation for 5 min. Arterial blood gas samples were obtained just before the onset and the termination of hypercapnia. Arterial pCO_2 increase during hypercapnia was ~ 20 mmHg (Table 1). LSF images were obtained every 30 s, for a total of 20 images. The first two images were taken as baseline, and the time course of CBF change during hypercapnia was calculated by drawing the margins of region of interest (ROI) around the entire hemisphere visible on speckle contrast image (Fig. 1, dotted line). When measurements were made using smaller ROIs (0.25 by 0.25 mm) placed over capillary bed or pial arterioles, the hypercapnic CBF increases did not differ from those recorded over the entire hemisphere, although the data showed higher variability. Peak CBF increase was taken as response amplitude. The rate of rise of CBF during hypercapnia (i.e. rising slope, %/min) was calculated as the peak CBF increase (%) divided by its latency from the onset of hypercapnia (min). The CO_2 reactivity index (%/mmHg) was calculated by dividing the peak CBF increase by the increase in arterial pCO_2 during hypercapnia.

Whisker stimulation

Whiskers were trimmed (5 mm) and manually stimulated vertically using a cotton tip applicator (5 Hz, 30 s). Whisker stimulation was started after the acquisition of the first image, which was taken as baseline. Laser speckle perfusion images were obtained every 7.5 s. The ROIs (0.25 by 0.25 mm) were placed within the whisker barrel field at the centre of regional hyperaemia (between 0 to 1 mm posterior, and 3.5 to 4.5 mm

lateral from bregma). Flow changes in capillary bed were measured by placing the ROI to avoid large pial vessels using speckle contrast images. In addition, arterial flow changes were selectively measured by placing the ROI on the middle cerebral artery branch supplying the whisker barrel cortex. The maximum increase in CBF during whisker stimulation was taken as response amplitude (% above baseline). Whisker stimulation and hypercapnia were typically tested once consecutively in the same animal.

Cortical spreading depression

(CSD) typically causes a characteristic triphasic CBF change in mice, with an initial transient vasoconstriction followed by a prolonged post-CSD oligemia (Ayata *et al.*, 2004b). We studied the vasoconstrictive effect of the spontaneous CSD triggered during distal middle cerebral artery occlusion using a micro-vascular clip (Ohwa Tsusho, Tokyo, Japan) (Shin *et al.*, 2006); this CSD evokes CBF changes in non-ischaemic cortex that are similar to KCl-induced CSDs, while allowing the imaging to be performed through intact skull (Ayata *et al.*, 2004b; Shin *et al.*, 2006). We placed a ROI (0.25 by 0.25 mm) within the non-ischaemic cortex avoiding large pial vessels (between 0.5 to 1.5 mm anterior, and 0.5 to 1.5 mm lateral to lambda; Fig. 1, circle). Only the first CSD after occlusion was studied in each experiment. We analysed CBF changes (% of baseline) by defining the onset of hypoperfusion as time zero, and measured the latency to and the amplitude of the following CBF deflection points: the onset and trough of hypoperfusion, peak of transient normalization, and 3 and 5 min after the onset of hypoperfusion. The CBF changes during CSD determined this way were plotted against time, and compared between strains, as well as between different age groups.

Resting CBF measurements

We used the inverse correlation time values ($1/\tau_c$) obtained from laser speckle analysis as a measure of resting CBF in arbitrary units, and compared this between groups. We have previously shown the validity and reproducibility of this approach in mice (Ayata *et al.*, 2004a). Although laser speckle flowmetry does not provide absolute values of CBF in ml/100 g/min, $1/\tau_c$ values (in arbitrary units) can be used to compare resting CBF among groups of mice (Ayata *et al.*, 2004a). Because age-related skull changes alter optical properties and resting $1/\tau_c$ values, comparisons can only be made for absolute resting $1/\tau_c$ values within the same age group, or for relative CBF changes among different age groups. Therefore, in this study we did not attempt to directly compare absolute resting CBF (i.e. $1/\tau_c$ values) among 3-, 8- and 19-month-old mice.

A β staining

At the end of the experiments, all brains were removed, and fresh frozen. The imaged right hemisphere was topically stained for A β using thioflavin S (0.001% in TBS, Sigma Aldrich, St Louis, MO). Briefly, brains were rinsed in $1 \times$ TBS for 10 min, immersed in thioflavin S for 20 min at 4°C and washed in $1 \times$ TBS for 10 min to remove unbound thioflavin S (Domnitz *et al.*, 2005).

Data analysis

The data were expressed as mean \pm standard deviation of mean. Statistical comparisons were made using paired or unpaired

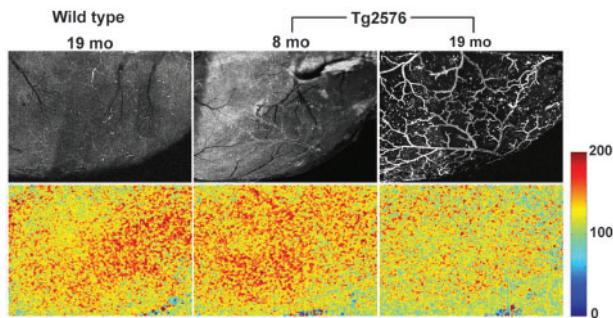


Fig. 2 Age-dependent cerebrovascular amyloid deposition and attenuation of hypercapnic hyperaemia in wild type and Tg2576 mice. Upper panel: Representative multiphoton images of 19-month-old wild type (left), and 8- and 19-month-old Tg2576 brains (middle and right, respectively) after topical thioflavin S staining. Only 19-month-old Tg2576 showed vascular A β deposition at this macroscopic level. Parenchymal A β plaques are also seen in 19-month-old Tg2576 as punctate labelling throughout the cortex at this low magnification. Lower panel: Representative pseudocolour LSF maps of CBF changes relative to baseline in 19-month-old wild type (left), and 8- and 19-month-old Tg2576 brain (middle and right, respectively) showing the global CBF increase after 5 min of 5% CO₂ inhalation. Colour bar shows CBF as percent of baseline. Hypercapnic hyperaemia was attenuated in 19-month-old Tg2576 compared to wild-type littermate.

Student's *t*-test, one-way ANOVA, or two-way ANOVA for repeated measures followed by Student–Newman–Keuls test. $P < 0.05$ was considered statistically significant.

Results

Topical thioflavin S-staining in 19-month-old Tg2576 mice ($n = 4$) revealed widespread and severe A β deposition in virtually all pial arterioles, but not veins, along with numerous A β plaques within the parenchyma. Such deposits were not present in 8-month-old Tg2576 ($n = 7$; see Fig. 2 for representative images).

Hypercapnic hyperaemia

Inhalation of 5% CO₂ for 5 min (Table 1) increased CBF globally (Fig. 2). Hypercapnic hyperaemia was attenuated in 19 but not 8-month-old Tg2576 mice (Figs. 2 and 3). The CO₂ reactivity index was reduced by more than 50% in the 19-month-old mutants compared to wild-type littermates (Fig. 3). Eight-month-old mutants did not differ from wild-type littermates. The slope of the response was also significantly attenuated in 19-month-old Tg2576 only (18 ± 12 versus $9 \pm 4\%/min$ in wild type and Tg2576,

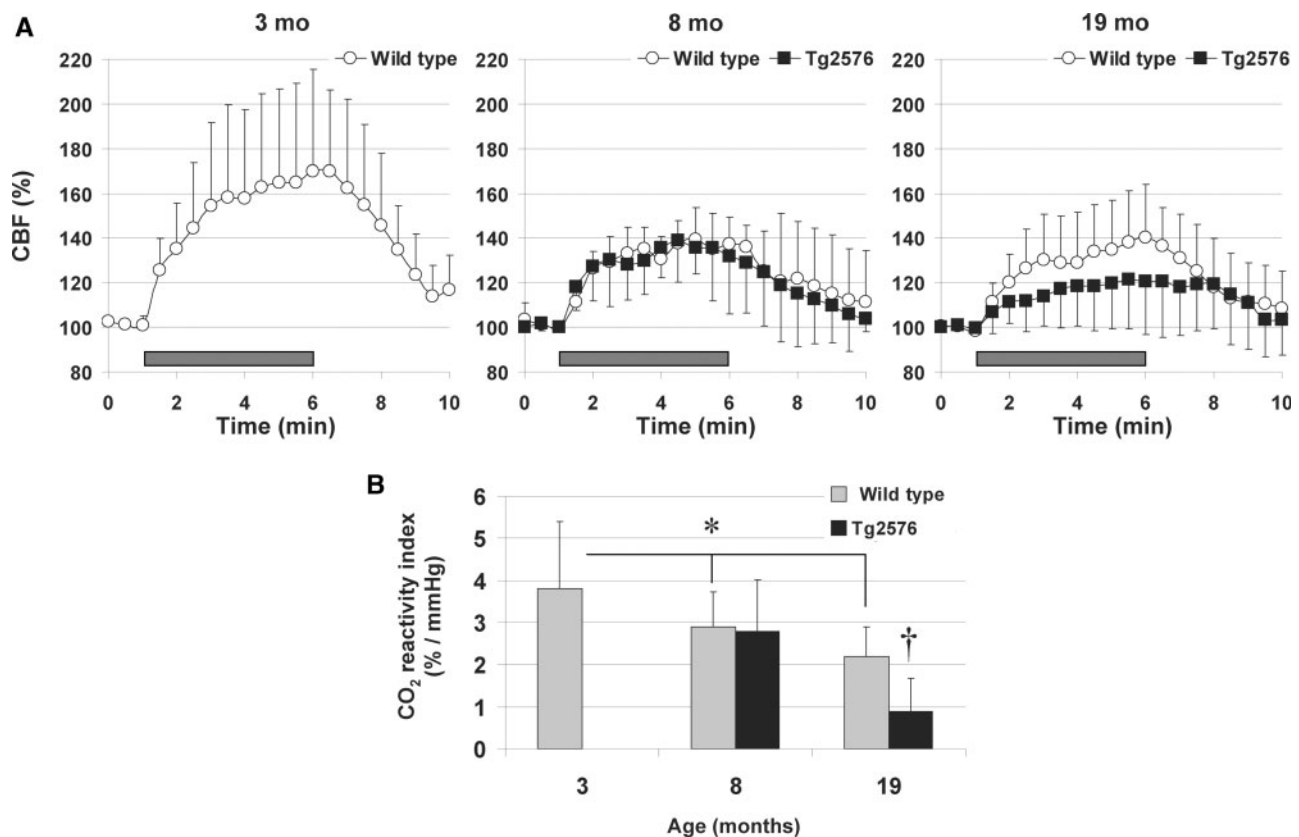


Fig. 3 Age-dependent attenuation of hypercapnic hyperaemia in wild type and Tg2576 mice. The CBF time course (A), and CO₂ reactivity index (B) during 5% CO₂ inhalation (horizontal bar) in 3-, 8- or 19-month-old wild type ($n = 5, 5$ and 16 , respectively), and 8- or 19-month-old Tg2576 mice ($n = 7$ and 8 , respectively). Hypercapnic hyperaemia was attenuated in 19 but not 8-month-old Tg2576 mice compared to wild-type littermates (\dagger , $P < 0.05$, 19-month-old Tg2576 versus 19-month-old wild type and 8-month-old Tg2576). Ageing alone also attenuated hypercapnic hyperaemia in wild-type mice ($*$, $P < 0.05$, one-way ANOVA). Vertical bars indicate \pm SD.

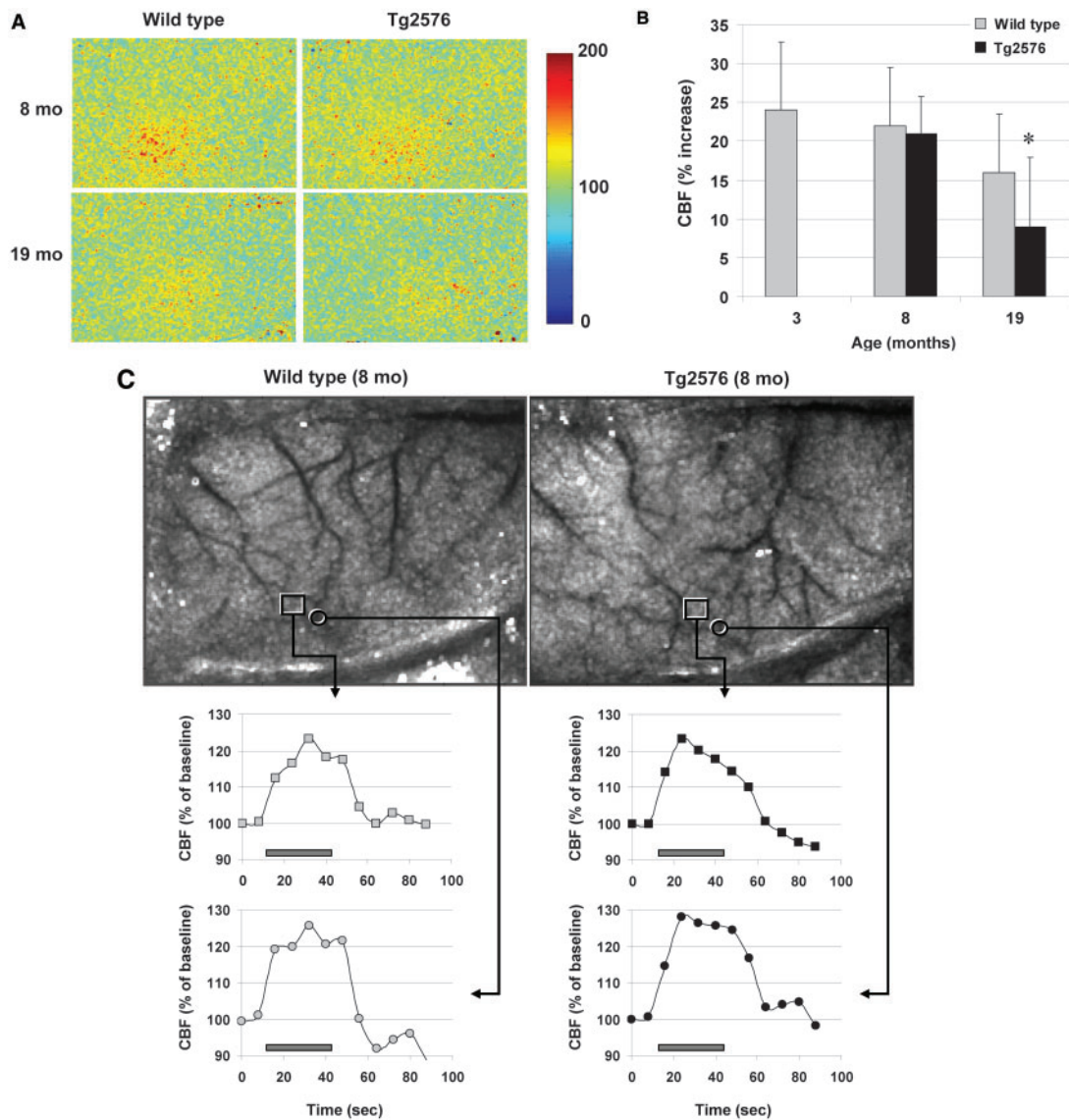


Fig. 4 Attenuation of whisker stimulation-induced hyperaemia in barrel cortex in aged Tg2576 mice. **(A)** Representative pseudocolour LSF maps of CBF changes within the whisker barrel field in 8- or 19-month-old wild type or Tg2576 mice. Colour bar represents CBF as percent of baseline. **(B)** Functional hyperaemia in whisker barrel cortex was attenuated in 19, but not 8-month-old Tg2576 compared to wild-type littermates (*, $P < 0.05$, 19-month-old Tg2576 versus 19-month-old wild type and 8-month-old Tg2576; $n = 8, 16$ and 7 , respectively). Ageing alone also tended to attenuate the functional hyperaemic response in wild-type mice ($P = 0.1$, one-way ANOVA among 3-, 8- and 19-month-old wild type; $n = 5, 5, 16$, respectively). Vertical bars indicate \pm SD. **(C)** Speckle contrast images from 8-month-old wild type and Tg2576 mice (upper panel) and representative time courses of functional CBF increase during 30 s whisker stimulation in the capillary bed and the middle cerebral artery branch supplying the barrel cortex (lower panel). These responses were preserved in 8-month-old Tg2576. The ROIs were placed to selectively measure CBF changes in the feeding artery (circle) and the capillary bed (square).

respectively; $P < 0.05$). Interestingly, ageing alone attenuated the response to hypercapnia in wild-type mice ($P < 0.05$; one-way ANOVA).

Whisker stimulation

Whisker stimulation increased CBF within the barrel cortex in all groups (Fig. 4A). The response was attenuated in 19, but not 8-month-old Tg2576 compared to wild-type littermates ($P < 0.05$, Fig. 4B). The attenuation in

19-month-old mutants was similar when the ROI was placed over an artery supplying the barrel cortex.

Cortical spreading depression

CSD is associated with an initial transient hypoperfusion (Fig. 5, h) followed by a longer-lasting post-CSD oligemia (Fig. 5, o), as previously described in mice (Ayata *et al.*, 2004b). Both the initial hypoperfusion and the subsequent post-CSD oligemia were attenuated in 19 but not 8-month-old Tg2576 compared to wild-type littermates, suggesting

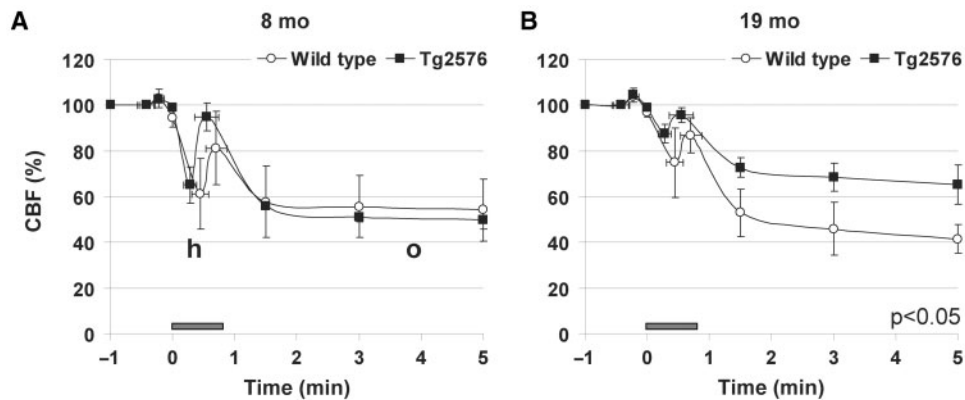


Fig. 5 The vasoconstrictive response to CSD was attenuated in 19 but not 8-month-old Tg2576 mice. The time course of CBF changes during CSD are shown in 8- (**A**) or 19-month-old (**B**) wild type and Tg2576 mice. CSD was associated with an initial transient hypoperfusion (h) followed by post-CSD oligemia (o). Both the initial transient hypoperfusion and long-lasting post-CSD oligemia were diminished in 19-month-old Tg2576 compared to wild-type littermates ($P < 0.05$; $n = 5$ and 8 , respectively); 8-month-old Tg2576 did not differ from wild type (two-way ANOVA for repeated measures; $n = 4$ and 5 , respectively). Horizontal gray bar shows the approximate timing of DC potential shift during CSD. Vertical and horizontal bars indicate SD of the amplitude of the CBF changes and the latency of the deflection point from the onset of hypoperfusion, respectively.

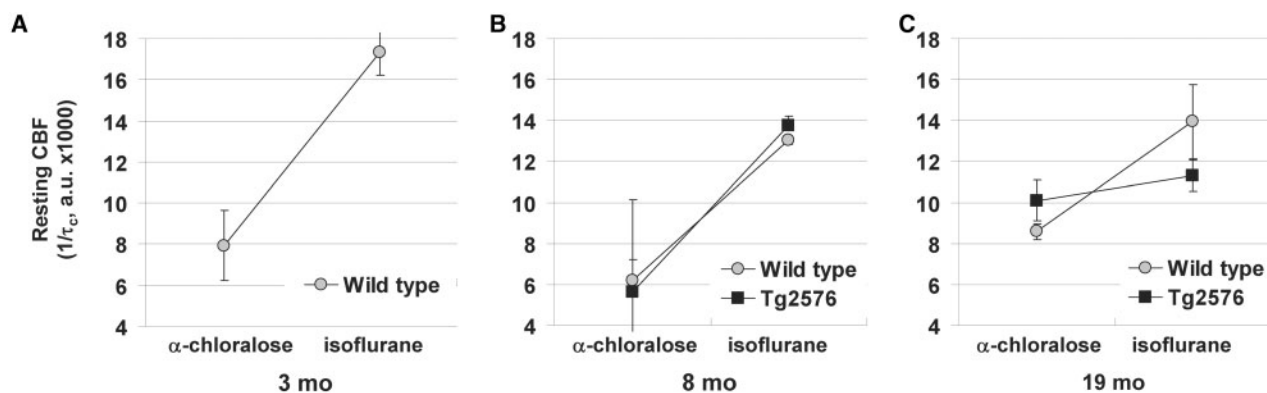


Fig. 6 The vasodilation to isoflurane and the vasoconstriction to α -chloralose anaesthesia were markedly attenuated in 19-month-old Tg2576. Graphs showing the effects of isoflurane and α -chloralose anaesthesia on resting CBF in wild type and Tg2576 mice. Resting CBF was estimated using the inverse correlation time value ($1/\tau_c$) obtained from speckle contrast analysis, and expressed in arbitrary units (a.u.). (**A**) In 3-month-old wild-type mice, resting CBF was 118% higher under isoflurane anaesthesia compared to α -chloralose ($n = 6$ and 9 , respectively; $P < 0.01$). (**B**) In 8-month-old wild type and Tg2576 mice, resting CBF was 110–143% higher under isoflurane anaesthesia compared to α -chloralose ($n = 5$ and 5 wild type, and 4 and 6 Tg2576, respectively; $P < 0.01$ between anaesthetics, $P = 0.66$ between strains, two-way ANOVA followed by Student–Newman–Keuls test). This difference was comparable to that in 3-month-old mice shown in (**A**). (**C**) In 19-month-old mice, the difference in resting CBF between isoflurane and α -chloralose anaesthesia was attenuated in wild type ($n = 8$ and 7 , respectively; $P < 0.01$ between anaesthetics), and abolished in Tg2576 ($n = 5$ and 5 , respectively; $P = 0.38$ between anaesthetics, $P = 0.02$ between strains) compared to 8-month-old mice. Vertical bars indicate \pm SD.

that the ability of cerebral vessels to constrict may also be impaired after amyloid deposition ($P < 0.05$, two-way ANOVA for repeated measures; Fig. 5). In wild-type mice, age alone did not affect the CBF response to CSD ($P = 0.45$, two way ANOVA for repeated measures among 3-, 8- and 19-month-old wild-type mice; data not shown), contrasting with the impact of aging on hypercapnic hyperaemia.

Resting CBF

We compared the effect of two general anaesthetics on resting CBF in wild type and Tg2576 mice using the inverse

correlation time ($1/\tau_c$) values obtained from laser speckle contrast analysis to estimate absolute blood flow (Ayata *et al.*, 2004a). In normal brain, isoflurane anaesthesia increases resting CBF by $\sim 30\%$, whereas α -chloralose reduces it by 30% (Szabo *et al.*, 1983; Okamoto *et al.*, 1997; Lenz *et al.*, 1998; Kehl *et al.*, 2002). We confirmed these findings in 3-month-old wild-type mice by showing that resting CBF doubled under isoflurane anaesthesia compared to α -chloralose ($P < 0.01$, respectively; Fig. 6A), consistent with previous observations (Ayata *et al.*, 2004a). In both 8-month-old Tg2576 mice and wild-type littermates, resting CBF was higher under isoflurane than

α -chloralose anaesthesia, and did not differ between strains ($P > 0.05$, wild type versus Tg2576; Fig. 6B). In 19-month-old wild-type mice, the difference in resting CBF between isoflurane and α -chloralose anaesthesia was attenuated compared to 3- and 8-month-old wild-type mice ($P < 0.05$; Fig. 6C). More importantly, resting CBF in 19-month-old mutants did not differ between the two anaesthetics ($P = 0.38$); both isoflurane-induced hyperaemia and α -chloralose-induced oligemia were diminished (Fig. 6C).

Discussion

Here, we confirm that vasomotor function is impaired in a transgenic mouse model of CAA, but show that this does not manifest itself before the overt deposition of amyloid in cerebral blood vessels under the stated experimental conditions. We tested the blood flow responses to diverse physiological and pharmacological stimuli that promote vasodilation (i.e. hypercapnia, whisker barrel field activation and isoflurane anaesthesia), and vasoconstriction (i.e. cortical spreading depression and α -chloralose anaesthesia), and showed that hyperaemic and oligemic responses are both impaired in 19-month-old mutant mice. Whether this vasomotor paralysis is due to decreased compliance or other mechanical properties of amyloid-laden vessels, or due to reduced reactivity or number of smooth muscle cells in advanced stages of CAA remains to be determined.

Vasodilatory dysfunction

Impaired cerebrovascular vasodilation to physiological (functional cortical activation, hypercapnia, hypotension) and pharmacological stimuli (acetylcholine, bradykinin, A23187) was previously shown in mouse models of CAA, developing as early as 2–3 months of age (Iadecola *et al.*, 1999; Niwa *et al.*, 2000a, b, 2002a; Park *et al.*, 2004, 2005). Using three distinct vasodilator stimuli, we found that cerebral hyperaemic responses were attenuated in 19-month-old Tg2576 mice with severe vascular amyloid deposition. In contrast, blood flow responses prior to vascular amyloid deposition (8 months) did not differ from wild-type littermates. We showed that flow changes were similarly attenuated in large pial arteries in 19 but not 8-month-old Tg2576, paralleling flow changes measured in brain parenchyma. Therefore, our data challenges the prevailing notion that elevated soluble A β is sufficient to cause cerebrovascular dysfunction.

Differences in experimental methodology may partly explain the discrepancy in the age of onset of impaired hypercapnic or functional hyperaemia in Tg2576 mice. Previous studies showing impaired vasodilator responses in 2–3-month-old CAA mutant models employed an open cranial window preparation (Zhang *et al.*, 1997; Iadecola *et al.*, 1999; Niwa *et al.*, 2000b, 2002a), compared to

imaging through an intact skull in the present study. Imaging the brain through an intact skull eliminates the possibility of injury during craniotomy and dural excision, exposure to air, cortical temperature fluctuations and altered intracranial pressure. It is possible that craniotomy and open skull preparation revealed a difference that became manifest perhaps via increased free radical production and endothelial dysfunction by A β (Iadecola *et al.*, 1999; Park *et al.*, 2004, 2005). More studies will be needed to clarify this point.

Our data suggest that mild to moderate elevation of A β levels in 8-month-old or younger Tg2576 mice are insufficient to impair cerebrovascular dilation to the tested physiological and pharmacological stimuli, and that the deposition of vascular amyloid may be a critical factor. Consistent with this, cerebral arteriolar dilation to topical pharmacological vasodilators acetylcholine- and sodium nitroprusside did not differ between 6-month-old Tg2576 mice and wild-type littermates, but was significantly attenuated in 14-month-old Tg2576 mice, when tested using a closed cranial window (Christie *et al.*, 2001). In another mouse model expressing human APP with Swedish double mutation under neuron-specific Thy-1 promoter (APP23) (Calhoun *et al.*, 1999; Burgermeister *et al.*, 2000; Winkler *et al.*, 2001), magnetic resonance angiography showed flow voids corresponding to slow or microturbulent flow, and corrosion casts demonstrated focal constriction, vessel elimination and rearrangement in major cerebral vessels in 60% of 20-month-old transgenic mice; none of the 11-month-old APP23 transgenic mice displayed any of these abnormalities (Beckmann *et al.*, 2003). In the same mouse model, hyperaemia following bicuculline- or acetazolamide was attenuated in 15-month-old or older mice showing deposits, but not in 8-month-old mice without amyloid deposition (Mueggler *et al.*, 2002). The APP23 model develops vascular amyloid deposition that is qualitatively and quantitatively comparable to Tg2576 (Calhoun *et al.*, 1999). Our data extends existing knowledge by non-invasively showing vasomotor paralysis (i.e. impaired dilation as well as constriction) to physiological stimuli, that does not become manifest before vascular amyloid deposition. However, not all mouse mutations causing A β deposition lead to vascular dysfunction. In mice with CAA overexpressing the London mutant of human APP (Moechars *et al.*, 1999), resting CBF and hypercapnic hyperaemia (7% CO₂ for 2 min) were similar to wild-type littermates even at 20–24 months of age (Van Dorpe *et al.*, 2000), suggesting that the relationship between vascular A β deposition and vasomotor dysfunction may be complex. It should be noted that different transgenic models have different kinetics of disease development, tissue A β increase and anatomic distribution of deposition, which makes direct comparisons across mouse models difficult.

Functional activation evoking the whisker barrel blood flow response tests the integrity of both vascular as well as parenchymal elements. It remains possible that the

attenuated CBF response to whisker stimulation in 19-month-old Tg2576 mice may be due to abnormal functional or metabolic activation of barrel cortex as a consequence of elevated A β levels and parenchymal plaques. Cerebral metabolism is not normal as evidenced by attenuated resting cerebral glucose utilization in 2–3-month-old Tg2576 mice (Niwa *et al.*, 2002*b*). Electrophysiologically, intrinsic membrane potential properties and overall synaptic innervation did not differ, although there was increased synaptic jitter, when neuronal responses from aged (>14 months) Tg2576 and wild-type littermates were compared (Stern *et al.*, 2004). Whether functional electrophysiological changes impact metabolic or vascular coupling mechanisms in Tg2576 remains to be determined. Regardless of its mechanisms, impaired vasodilation in advanced CAA may predispose to chronic or episodic metabolic mismatch thereby contributing to parenchymal neuropathology in AD.

Vasoconstrictive dysfunction

Our data also suggest that arterial constriction is also impaired during CSD or under α -chloralose anaesthesia in 19 but not 8-month-old Tg2576. These two stimuli constrict cerebral vessels via different mechanisms. The CBF response to CSD in mice is characterized by a transient and profound vasoconstriction during the DC shift, that is believed to be directly mediated by elevated extracellular potassium concentration (50 mM or more) (Godfraind *et al.*, 1986; Ayata *et al.*, 2004*b*; Windmuller *et al.*, 2005; Shin *et al.*, 2006). This is followed by a longer lasting post-CSD oligemia (Fig. 5) which may involve vasoconstrictive prostanoids (Shibata *et al.*, 1992). α -Chloralose, on the other hand, is believed to reduce CBF via suppression of cerebral metabolism (Sandor *et al.*, 1977; Nakao *et al.*, 2001). Hence, our data demonstrate cerebral vasoconstrictive dysfunction, regardless of the mediators and mechanisms involved, which develops only after vascular amyloid deposition in aged Tg2576. This is clinically relevant because impaired vasoconstriction may reduce the ability of cerebral arterioles to dampen sudden surges in microcirculatory perfusion pressure during hypertensive episodes, and predispose to intracerebral haemorrhages.

Correlation of pathology and cerebrovascular dysfunction

We chose to study Tg2576 mice at 8 and 19 months of age in order to test the impact of elevated A β without CAA and with severe CAA, respectively. Vessels from 8-month-old Tg2576 mice do not harbour amyloid deposits, whereas in 19-month-old Tg2576 more than 90% of pial arteries and arterioles are heavily laden with amyloid deposits (Fig. 2), with loss of smooth muscle cells (Domnitz *et al.*, 2005; Robbins *et al.*, 2006). In contrast, parenchymal soluble A β levels are already elevated at 2–3 months of age (Niwa *et al.*, 2000*b*), and increase exponentially starting around

6–7 months (Kawarabayashi *et al.*, 2001). Our data suggest that elevated soluble A β alone without deposition is insufficient to cause vasomotor dysfunction as tested herein. Because soluble A β_{40} and A β_{42} levels continue to rise between 8 and 19 months of age, and 19-month-old Tg2576 mice also harbour parenchymal plaques, they may contribute to the vasomotor dysfunction in 19-month-old Tg2576 mice.

In humans, the incidence of CAA increases with age; half of all individuals over age 70 years and 80–90% of AD patients have varying degrees of CAA (Mandybur, 1975; Vinters, 1987; Ellis *et al.*, 1996). Cerebral amyloid angiopathy often becomes symptomatic in patients with severe vascular A β deposition, usually after age 70 years. Although recurrent lobar haemorrhages are the typical presenting feature, patients with CAA also have a higher risk of developing ischaemic stroke, and leukoencephalopathy (Olichney *et al.*, 1995, 1997; Greenberg, 2002). Cerebral amyloid angiopathy has been proposed as an exacerbating factor for cognitive dysfunction in AD based on data from population studies and familial CAA syndromes (Snowdon *et al.*, 1997; Grabowski *et al.*, 2001; Natte *et al.*, 2001; Pfeifer *et al.*, 2002). However, it is unclear whether the negative impact of CAA on cognition in AD patients simply reflects the cumulative effect of cerebral infarcts, or whether CAA causes chronic cerebrovascular dysregulation in the absence of overt infarction. The latter view is supported by experimental data showing vascular impairment by A β and in transgenic mouse models of AD and CAA (Thomas *et al.*, 1996; Crawford *et al.*, 1998; Iadecola *et al.*, 1999; Niwa *et al.*, 2000*a*, 2001; Paris *et al.*, 2003). Indeed in some studies vascular dysfunction manifested long before the development of CAA, suggesting that elevated A β levels may be sufficient to disrupt vascular function (Zhang *et al.*, 1997; Iadecola *et al.*, 1999; Niwa *et al.*, 2000*b*, 2002*a*, *b*). However, pathologically demonstrated cerebrovascular amyloid deposition is an important independent risk factor for cognitive impairment (odds ratio 9.3) (2001; Greenberg *et al.*, 2004; Greenberg, 2006) as well as subcortical white matter disease (Haglund and Englund, 2002). The data shown in the Tg2576 are consistent with this notion, and suggest that A β impacts cerebrovascular function only after vascular deposition. Whether vascular deposition is also a prerequisite for cerebrovascular dysfunction in patients with AD remains to be tested.

Effects of ageing on cerebrovascular function

We observed that aged wild-type mice also displayed attenuated hypercapnic hyperaemia, metabolic coupling and anaesthetic influences on resting CBF. These data should be interpreted with caution because imaging was performed through intact skull, and age-dependent changes in skull properties might have influenced the relative CBF changes recorded. However, hypercapnic hyperaemia was attenuated in wild-type mice only between 3 and 8 months

of age, whereas functional hyperaemia was attenuated only between 8 and 19 months of age; CSD-induced CBF changes did not differ among the age groups. Hence, our data suggest that the age-dependent cerebrovascular dysfunction in these experiments is not merely a non-specific and uniform attenuation of all 'measured' relative CBF responses due to age-dependent changes in optical properties of the skull. The impact of ageing on cerebrovascular function was previously studied in both cerebral and systemic vessels. For example, endothelium-dependent cerebral vasodilation was significantly reduced in aged rats (22–24 mo) compared to young adult rats (6–8 mo) when studied using closed cranial windows (Mayhan *et al.*, 1990). More recently, functional hyperaemia, and acetylcholine- and bradykinin-induced CBF increases were age-dependently attenuated in 12- and 24-month-old wild-type mice (Park *et al.*, 2006). Our data confirm these reports, and underscore the importance of age when studying the impact of slowly progressive cerebrovascular diseases of ageing on CBF regulation.

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