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Cerebrovascular autoregulation is profoundly impaired in mice overexpressing amyloid precursor protein

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Niwa, Kiyoshi, Ken Kazama, Linda Younkin, Steven G. Younkin, George A. Carlson, and Costantino Iadecola. Cerebrovascular autoregulation is profoundly impaired in mice overexpressing amyloid precursor protein. *Am J Physiol Heart Circ Physiol* 283: H315–H323, 2002. First published March 7, 2002; 10.1152/ajpheart.00022.2002.—The amyloid-β (Aβ) peptide, which is derived from the amyloid precursor protein (APP), is involved in the pathogenesis of Alzheimer's dementia and impairs endothelium-dependent vasodilation in cerebral vessels. We investigated whether cerebrovascular autoregulation, i.e., the ability of the cerebral circulation to maintain flow in the face of changes in mean arterial pressure (MAP), is impaired in transgenic mice that overexpress APP and Aβ. Neocortical cerebral blood flow (CBF) was monitored by laser-Doppler flowmetry in anesthetized APP(+) and APP(−) mice. MAP was elevated by intravenous infusion of phenylephrine and reduced by controlled exsanguination. In APP(−) mice, autoregulation was preserved. However, in APP(+) mice, autoregulation was markedly disrupted. The magnitude of the disruption was linearly related to brain Aβ concentration. The failure of autoregulation was paralleled by impairment of the CBF response to endothelium-dependent vasodilators. Thus Aβ disrupts a critical homeostatic mechanism of the cerebral circulation and renders CBF highly dependent on MAP. The resulting alterations in cerebral perfusion may play a role in the brain dysfunction and periventricular white-matter changes associated with Alzheimer's dementia.

Alzheimer's disease; cerebral blood flow; endothelium-dependent vasodilation

ALZHEIMER'S DISEASE (AD) is a highly prevalent form of dementia characterized neuropathologically by amyloid deposition in neuropil (amyloid plaques) and cerebral blood vessels (amyloid angiopathy) and by alterations in phosphorylated neurofilament, which is termed neurofibrillary tangles (see Refs. 33 and 39 for a review). Recent advances in the molecular pathology of AD have provided evidence that implicates the amyloid precursor protein (APP), a transmembrane glycoprotein, in the mechanisms of the disease (33). Peptides produced by proteolytic processing of APP, the Aβ peptides, are major constituents of vascular (Aβ1–40) and parenchymal (Aβ1–42) amyloid (5, 17), whereas mutations in the APP gene are linked to familial forms of AD (33). Transgenic mice that overexpress APP exhibit elevated brain levels of Aβ and develop neuropathological, cognitive, and cerebral metabolic alterations which resemble those of AD (e.g., see Refs. 9, 29). Therefore, a widely held hypothesis concerning the pathogenesis of AD is that abnormal processing of APP results in accumulation of Aβ in the brain, which in turn leads to neuronal dysfunction and neurodegeneration (33).

The mechanisms by which Aβ leads to brain dysfunction have not been elucidated in full (see Ref. 35 for a review). Although there is evidence that Aβ alters neuronal function (23), recent data suggests that this peptide can also produce cerebrovascular dysfunction. Thus synthetic Aβ impairs endothelium-dependent relaxation and enhances vasoconstriction both in vivo and in vitro (27, 30, 38). Furthermore, elevations of brain Aβ levels in APP mice are associated with a reduction in resting cerebral blood flow (CBF) and an impairment of selected vasodilatatory responses of the cerebral circulation (13, 29, 31). The functional implications of these cerebrovascular alterations have not been fully elucidated, and their impact on the mechanisms regulating the cerebral circulation remains to be defined.

Cerebrovascular autoregulation is one of the fundamental properties of the cerebral circulation through which CBF is maintained relatively constant despite variations in mean arterial pressure (MAP) within a certain range (7). This property of cerebral blood vessels acts as a critical homeostatic mechanism that assures stable brain perfusion during the fluctuations in MAP that occur during normal activities (14) and in pathological states associated with hypotension or hypertension (32). Considering that the brain is critically dependent on a stable blood supply, alterations in autoregulation can have deleterious effects on the structural and functional integrity of the brain (22). We report here that mice that overexpress APP have a profound disruption of cerebrovascular autoregulation that is more pronounced in transgenic lines with

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higher levels of brain Aβ. The loss of autoregulation is paralleled by alterations in endothelium-dependent cerebrovascular responses. Although the findings unveil a previously unrecognized effect of APP and Aβ overexpression on cerebrovascular function, they also raise the possibility that failure of autoregulation may contribute to brain dysfunction in diseases such as AD in which brain Aβ is elevated.

METHODS

Transgenic Mice

The transgenic lines used in these studies have been described previously (31). All mice were studied at age 2–3 mo. All APP transgenes consist of the human APP695 isoform expressed using the cosTet hamster prion protein (PrP)-derived cosmid (10). Tg6209 is wild-type APP whereas Tg2123 has the "Swedish" K670N, M671L changes; both of these arrays have a 3′-*myc* epitope tag. The Tg6209 and Tg2123 transgene arrays are expressed on the inbred FVB/N background (10). Tg2576 mice express Swedish mutant APP without a *myc* tag on a mixed C57BL/6J-SJL/J background (9, 10). Results from Tg2123 male (M) and female (F) mice are presented separately because the transgene array in this line is located on the X chromosome. Owing to random X inactivation, only half of the somatic cells in female mice will express the transgene; therefore, males express approximately twice the amount of APP as females. Amyloid plaques and vascular amyloid are not present in Tg6209, Tg2123F, or Tg2123M mice (10). The line Tg2576 does not exhibit amyloid deposition at the age at which the mice were studied (2–3 mo) (9). For all lines studied, APP(+) mice were compared with corresponding APP(−) age-matched littermates.

CBF Study by Laser-Doppler Flowmetry

Techniques used for studying CBF in mice by laser-Doppler flowmetry (LDF) were similar to those previously described (13, 30). Mice were anesthetized with urethane (750 mg/kg ip) and chloralose (50 mg/kg ip). The trachea was intubated and mice were artificially ventilated with an oxygen-nitrogen mixture. One femoral artery was cannulated for arterial pressure recording and blood sampling. Rectal temperature was maintained at 37°C using a thermostatically controlled rectal probe connected to a heating lamp. End-tidal CO_2 was monitored by a CO_2 analyzer (Capstar-100, CWI) (13). A small craniotomy (2 × 2 mm) was performed to

Table 1. *Arterial pressure and blood gases in APP transgenic mice*

APP transgenic lines	*n*	MAP, mmHg	pH	P_{CO_2}, mmHg	P_{O_2}, mmHg
Cerebrovascular autoregulation					
Tg6209 (−)	12		7.31 ± 0.02	35.4 ± 0.5	129.5 ± 5.2
Tg6209 (+)	10		7.29 ± 0.01	35.5 ± 0.4	138.6 ± 4.9
Tg2123F (−)	12		7.30 ± 0.01	33.8 ± 0.8	136.0 ± 5.4
Tg2123F (+)	8		7.30 ± 0.01	35.0 ± 0.8	137.1 ± 2.5
Tg2123M (−)	8		7.28 ± 0.03	35.0 ± 0.2	118.6 ± 3.3
Tg2123M (+)	8		7.32 ± 0.03	33.2 ± 0.4	130.0 ± 4.3
Tg2576 (−)	10		7.31 ± 0.02	33.9 ± 0.6	138.7 ± 4.3
Tg2576 (+)	9		7.31 ± 0.02	34.0 ± 0.5	130.2 ± 4.1
Endothelium-dependent and -independent cerebrovascular responses					
ACh, BK, A-23187, U-46619, SNAP					
Tg6209 (−)	6	99 ± 4	7.31 ± 0.01	34.5 ± 0.5	130.6 ± 6.4
Tg6209 (+)	6	101 ± 3	7.30 ± 0.02	34.3 ± 1.0	127.4 ± 8.3
Tg2123F (−)	6	96 ± 2	7.30 ± 0.01	33.8 ± 0.8	136.0 ± 5.4
Tg2123F (+)	6	98 ± 2	7.30 ± 0.01	35.0 ± 0.9	132.1 ± 6.7
Tg2123M (−)	5	96 ± 3	7.31 ± 0.02	34.7 ± 0.9	131.1 ± 7.9
Tg2123M (+)	5	102 ± 3	7.36 ± 0.02	34.1 ± 0.7	127.6 ± 2.3
Tg2576 (−)	6	96 ± 3	7.31 ± 0.01	34.2 ± 0.6	134.6 ± 4.1
Tg2576 (+)	6	93 ± 3	7.31 ± 0.02	36.0 ± 0.7	120.3 ± 5.2
Hypercapnia					
Tg6209 (−)	6†	92 ± 4	7.17 ± 0.02*	57.1 ± 3.8*	127.4 ± 7.8
Tg6209 (+)	6†	91 ± 2	7.15 ± 0.02*	55.1 ± 1.8*	124.3 ± 8.8
Tg2123F (−)	6†	87 ± 5	7.10 ± 0.02*	53.4 ± 0.6*	132.7 ± 2.5
Tg2123F (+)	6†	90 ± 7	7.12 ± 0.04*	55.0 ± 0.5*	144.7 ± 4.7
Tg2123M (−)	6†	89 ± 7	7.15 ± 0.04*	54.0 ± 1.3*	127.8 ± 3.1
Tg2123M (+)	6†	89 ± 4	7.18 ± 0.05*	53.3 ± 1.7*	135.2 ± 3.5
Tg2576 (−)	6†	91 ± 5	7.16 ± 0.02*	54.2 ± 0.9*	129.1 ± 3.9
Tg2576 (+)	6†	89 ± 4	7.15 ± 0.02*	55.5 ± 0.9*	136.0 ± 3.3
Hypocapnia					
Tg6209 (−)	6‡	89 ± 3	7.44 ± 0.04*	20.8 ± 1.8*	130.0 ± 4.7
Tg6209 (+)	6‡	92 ± 3	7.43 ± 0.03*	21.7 ± 1.2*	121.7 ± 8.2
Tg2123F (−)	6‡	91 ± 3	7.42 ± 0.01*	20.8 ± 0.7*	130.2 ± 8.2
Tg2123F (+)	6‡	98 ± 4	7.43 ± 0.01*	19.4 ± 1.0*	127.1 ± 6.6
Tg2123M (−)	5‡	93 ± 3	7.45 ± 0.01*	21.4 ± 1.3*	130.8 ± 5.9
Tg2123M (+)	5‡	102 ± 6	7.47 ± 0.02*	19.5 ± 1.3*	126.8 ± 3.6
Tg2576 (−)	6‡	93 ± 3	7.45 ± 0.02*	21.5 ± 0.9*	129.5 ± 6.0
Tg2576 (+)	6‡	102 ± 4	7.48 ± 0.02*	20.3 ± 1.7*	136.1 ± 6.5

Values are means ± SE; *n*, no. of mice. APP, amyloid precursor protein. *$P < 0.05$ from respective control (ANOVA and Tukey's test); †cerebrovascular autoregulation and hypercapnia were tested in the same mice. ‡ACh, bradykinin (BK), A-23187, U-46619, *S*-nitroso-*N*-acetylpenicillamine (SNAP), and hypocapnia were tested in the same mice.

expose the whisker-barrel area of the somatosensory cortex, the dura was removed, and the site was superfused with Ringer solution; temperature was 37°C and pH was 7.3–7.4 (30). The LDF probe (tip diameter 0.8 mm, Vasamedic; St. Paul, MN) was mounted on a micromanipulator (Kopf) and positioned 0.5 mm above the pial surface. Zero values for CBF were obtained after the heart was stopped by an overdose of halothane at the end of the experiment.

Determination of Aβ

Aβ measurement by ELISA has been described in detail previously (37). At the end of the experiments, the hemisphere contralateral to the craniotomy was sonicated in formic acid and centrifuged. The formic acid extract was neutralized and assayed by ELISA using BAN50 as capture for human transgene-specific Aβ and detection with BA27 for Aβ1–40 and BC05 for Aβ1–42. Femtomoles per milliliter were calculated by comparing the sample absorbance to the absorbance of a standard curve. Values were corrected with the wet weight of the original homogenate and are finally expressed as picomoles per gram of wet weight.

Experimental Protocols

Cerebrovascular autoregulation. Techniques used for studying cerebrovascular autoregulation in rodents were similar to those previously described (11, 16). Mice were anesthetized and prepared for CBF measurement by LDF. After stabilization of MAP and blood gases (Table 1), MAP was elevated or decreased in 10-mmHg steps by intravenous infusion of phenylephrine ($1–2\ \mu g \cdot kg^{-1} \cdot min^{-1}$) or via controlled exsanguination (100–400 μl of arterial blood), respectively (16). The range of MAP studied was 20–160 mmHg. CBF values were recorded 5 min after MAP was changed. Lower and upper limits of autoregulation were tested in separate animals (16) because of potential pathological effects of changes in MAP above or below the autoregulated range. At the end of the experiments, brains were removed and frozen in liquid nitrogen for subsequent measurement of Aβ.

Endothelium-dependent and -independent responses. After stabilization of MAP and blood gases (Table 1), ACh (10 μM, Sigma), bradykinin (BK, 50 μM, Sigma), the calcium ionophore A-23187 (3 μM, Sigma), *S*-nitroso-*N*-acetylpenicillamine (SNAP, 100 or 500 μM, RBI), or the thromboxane analog U-46619 (0.1 or 1 μM, Sigma) were superfused on the cerebral cortex until the evoked change in CBF reached a steady state (usually 3–5 min). The concentrations of ACh, BK, and A-23187 were chosen to produce 50% of maximal responses as determined by dose-response curves (13). In the mouse microcirculation, the CBF response to ACh is mediated by endothelial nitric oxide (NO), whereas responses to BK and A-23184 are mediated by cyclooxygenase-1 through reactive oxygen species (ROS; see Refs. 28, 36). To study the changes in CBF produced by systemic hypercapnia, CO_2 was introduced in the circuit of the ventilator until arterial P_{CO_2} (Pa_{CO_2}) reached 50–60 mmHg (see Table 1). The response to hypercapnia is not dependent on the endothelium, and NO is thought to play a "permissive" role in the vasodilation (12, 40). Hypocapnia (Pa_{CO_2} = 19–22 mmHg) was produced by increasing the ventilatory rate of the respirator. At the end of the experiments, brains were removed and frozen in liquid nitrogen for subsequent measurement of Aβ.

Data Analysis

Data in text and figures are expressed as means ± SE. Two-group comparisons were analyzed by the two-tailed *t*-test for independent samples. Multiple comparisons were evaluated by ANOVA and Tukey's test. Probability values <0.05 were considered statistically significant.

RESULTS

Cerebrovascular Autoregulation in APP Transgenics

In these experiments, we studied cerebrovascular autoregulation in four lines of APP transgenic mice that expressed different levels of Aβ in brain. Lowest Aβ levels were observed in the Tg6209 line, and highest levels were identified in the Tg2576 line (Fig. 1*A*). In APP(−) mice, CBF was independent of MAP in the range of 60–120 mmHg, which indicates that autoregulation was present (Fig. 2*A*). No differences in auto-

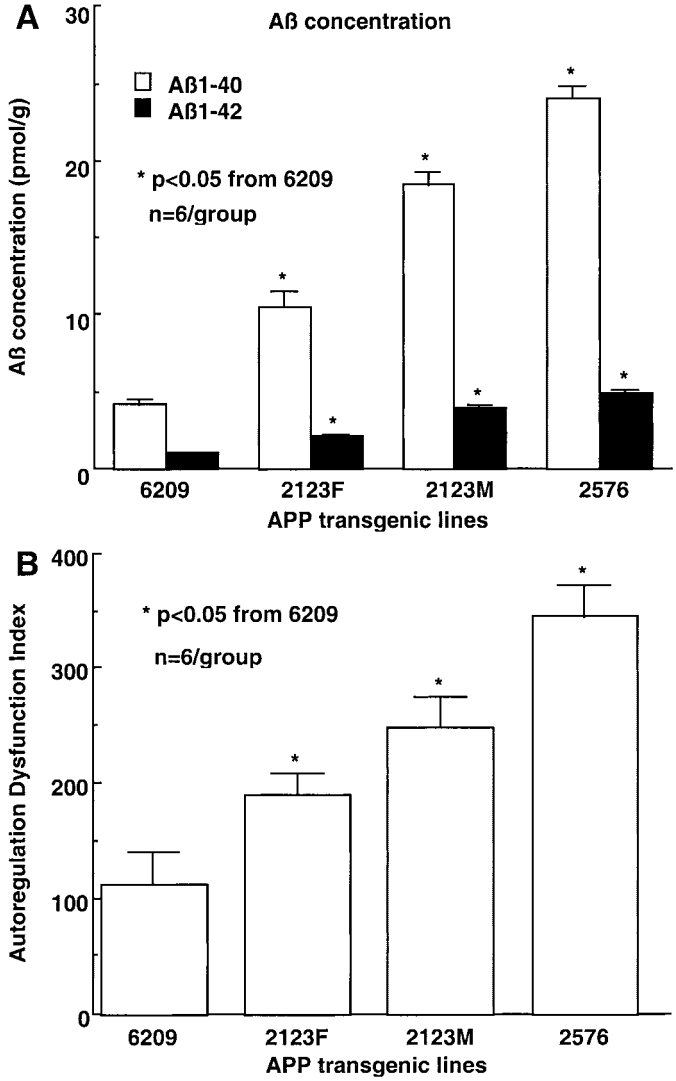

Fig. 1. *A*: brain concentrations of amyloid-β protein isoforms Aβ1–40 and Aβ1–42 in the transgenic lines studied. *B*: autoregulation dysfunction index (ADI) in the different transgenic lines studied. For each transgenic line, ADI is computed by subtracting cerebral blood flow (CBF, in percent change) in amyloid precursor protein (APP)− mice from CBF in APP(+) mice at each level of mean arterial pressure (MAP). Absolute difference is then summed for all MAP levels. *$P < 0.05$, ANOVA and Tukey's test.

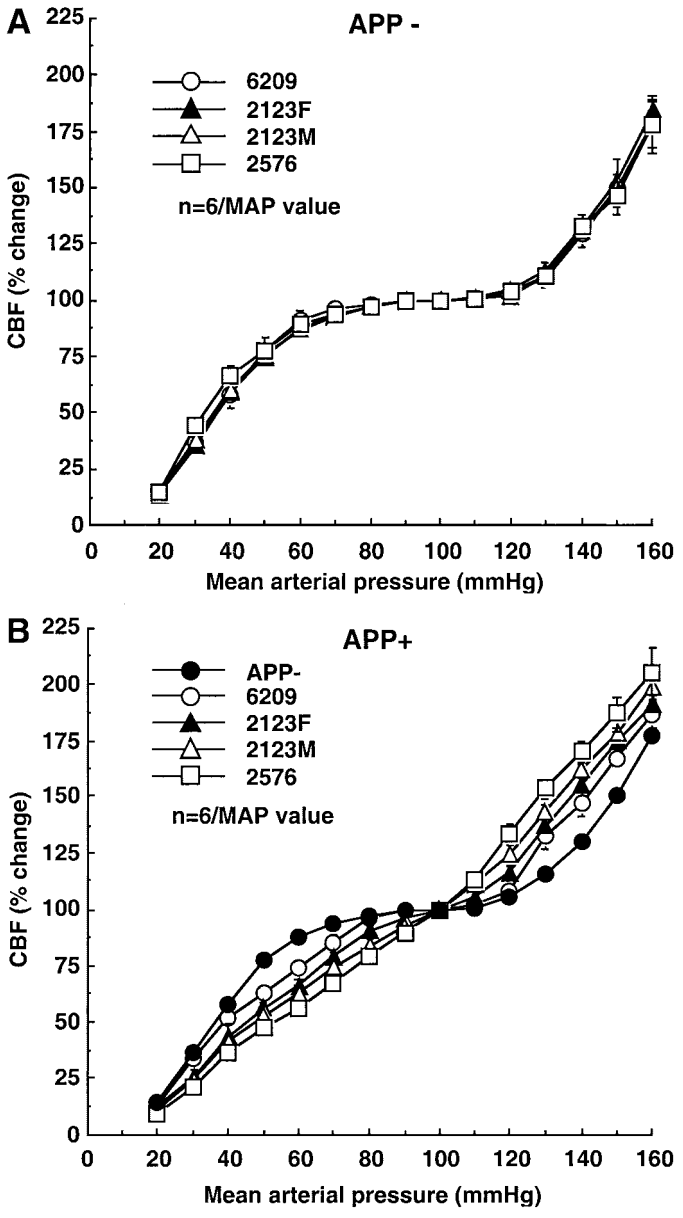

Fig. 2. *A*: relationship between CBF and MAP in different lines of APP(−) mice. In all transgenic lines, CBF is statistically different from the value at 100 mmHg at MAP values of 20–50 and 140–160 mmHg ($P < 0.05$, ANOVA). *B*: relationship between CBF and MAP in different lines of APP(+) mice. MAP values at which CBF is statistically different from respective APP(−) group are Tg6209: 50–70, 130–160; Tg2123F: 30–70, 120–160; Tg2123M: 30–80, 120–160; and Tg2576: 30–90, 110–160 mmHg ($P < 0.05$, ANOVA).

regulation were observed among APP(−) mice of different genetic backgrounds (Fig. 2*A*). In APP(+) mice, however, the relationships between CBF and MAP were substantially altered (Fig. 2*B*). There was a progressive reduction in the autoregulated range, which was more marked in transgenic lines with higher levels of Aβ (Fig. 2*B*). In Tg2576 mice, the relationship between MAP and CBF was essentially linear, which indicates total dependence of CBF on MAP and loss of autoregulation (Fig. 2*B*).

To quantify the impairment of autoregulation and to correlate it with brain Aβ levels, we devised an autoregulation dysfunction index (ADI). For any given transgenic line, ADI is defined as the sum of the absolute difference between CBF in APP(−) mice and APP(+) mice at each level of MAP (Fig. 2). We used the ADI instead of the autoregulation index (e.g., Ref. 2) to quantify disruption in both the upper and lower ranges of the curve. ADI was lowest in Tg6209 mice and highest in Tg2576 mice, which indicates a relationship between Aβ levels and dysfunction of autoregulation (see Fig. 1*B*). We then correlated the ADI with the level of brain Aβ measured postmortem in each mouse. The relationship between Aβ1–40 or Aβ1–42 and ADI was linear and highly correlated ($r^2 = 0.904$ for Aβ1–40; $P < 0.001$; Fig. 3). These findings indicate that the autoregulatory dysfunction in APP mice is proportional to brain Aβ levels.

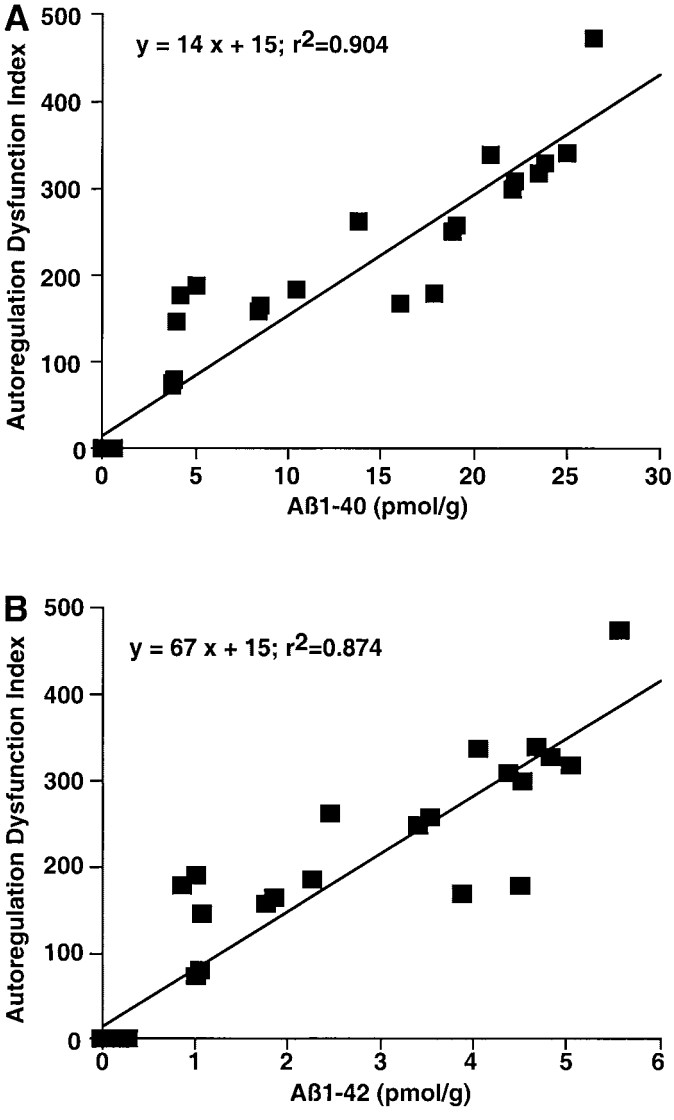

Fig. 3. Correlations between autoregulation dysfunction index and brain concentrations of Aβ1–40 (*A*) and Aβ1–42 (*B*).

Vasodilatatory and Vasoconstrictor Responses in APP Transgenics

Autoregulation depends on the ability of cerebral resistance vessels to dilate when MAP falls and to constrict when MAP rises (7). To determine whether the dysfunction in autoregulation of APP mice was related to alterations in cerebrovasodilation, we studied cerebrovascular reactivity to endothelial-dependent and -independent vasodilators. The increase in CBF produced by the endothelium-dependent vasodilators ACh, BK, and A-23187 were attenuated in APP mice. The effect was greater in mice with higher levels of brain Aβ (Fig. 4). In contrast, responses to the endothelium-independent vasodilator SNAP and to hypercapnia did not differ between APP(−) and APP(+) mice except in the Tg2576 line, in which a reduction was observed (Figs. 4*D* and 5).

To determine whether the dysfunction of autoregulation was related to an impairment of vasoconstriction, we studied cerebrovascular reactivity to interventions that reduce CBF. The thromboxane analog U-46619 reduced CBF more in APP(+) mice, and the effect was greater in transgenic lines with higher Aβ levels (Fig. 6*A*). On the other hand, hypocapnia (Pa_{CO_2} = 18–22 mmHg) reduced CBF comparably in APP(+) and APP(−) mice (Fig. 6*B*). Therefore, the reactivity of CBF to vasoconstrictor stimuli is not impaired in APP mice.

DISCUSSION

We have demonstrated that mice overexpressing APP exhibit a marked disruption in cerebrovascular autoregulation. The effect is greatest in Tg2576 mice in which autoregulation is essentially lost. To determine

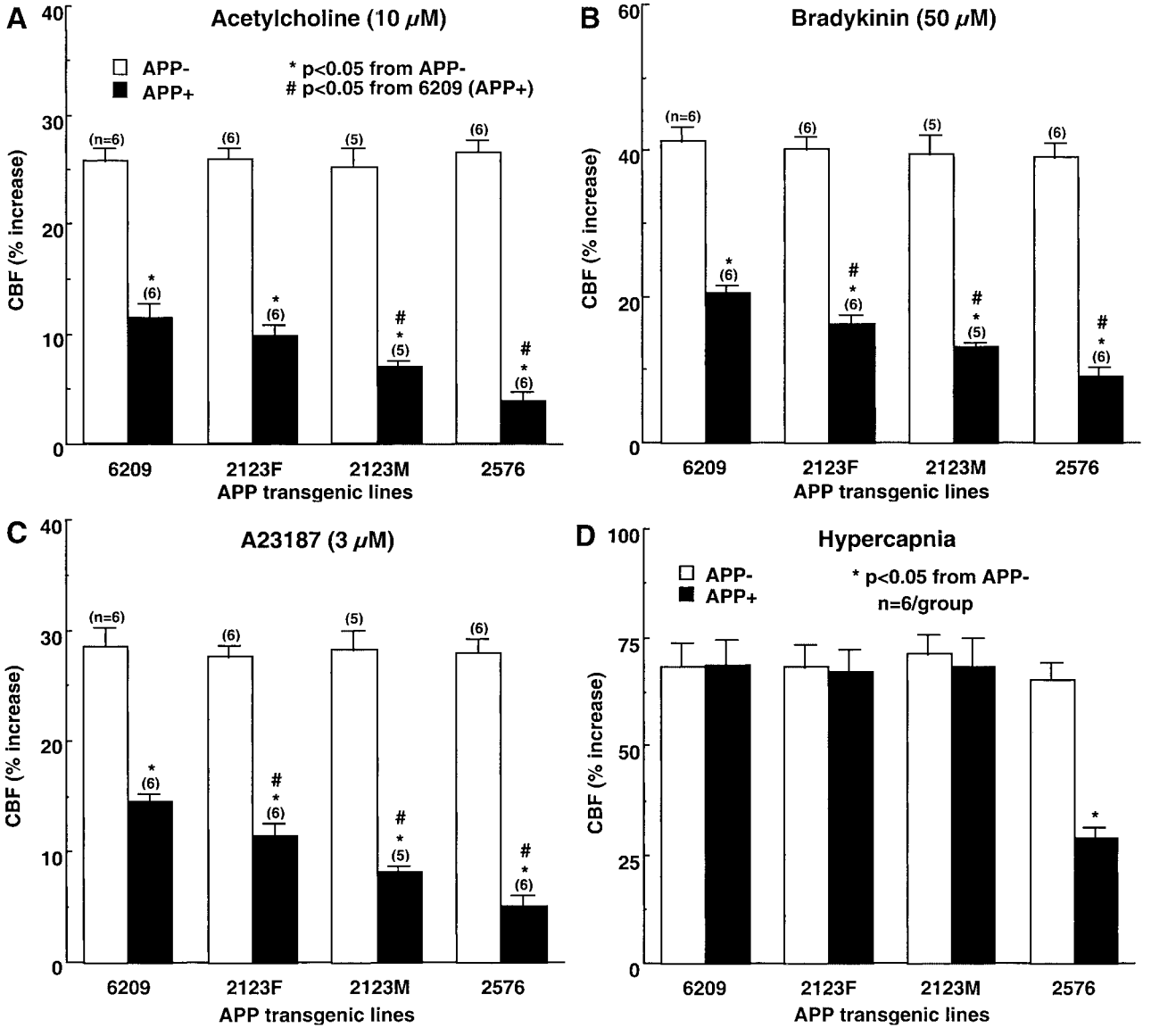

Fig. 4. Increases in CBF produced by ACh (*A*), bradykinin (*B*), A-23187 (*C*), and hypercapnia (*D*) in the different transgenic lines studied. *$P < 0.05$, *t*-test; #$P < 0.05$, ANOVA and Tukey's test.

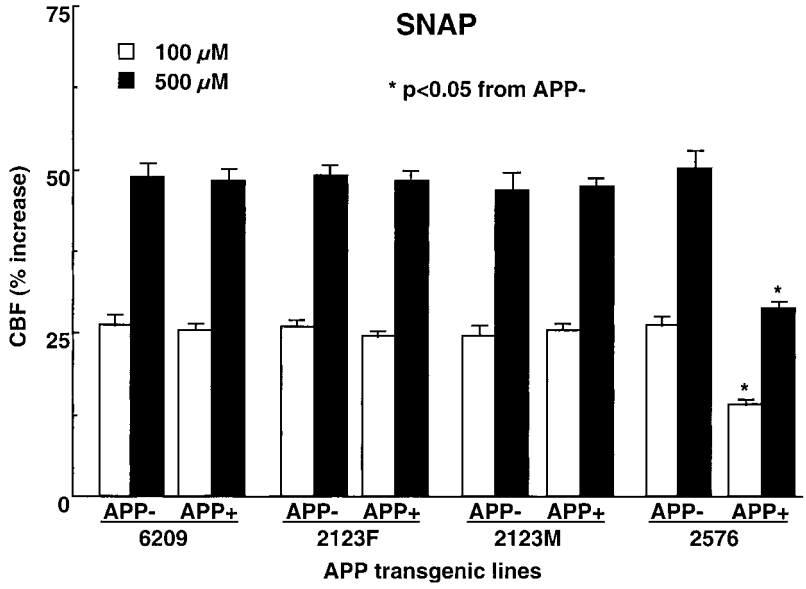

Fig. 5. Increase in CBF produced by two concentrations of the nitric oxide donor *S*-nitroso-*N*-acetylpenicillamine (SNAP) in the different transgenic lines studied. $^*P < 0.05$, *t*-test.

whether the mechanisms of the disruption were related to brain Aβ levels, we devised the ADI, and we correlated this index with brain Aβ measured in each mouse in which autoregulation was studied. It was found that there is a linear correlation between ADI and brain Aβ1–40 and Aβ1–42. Thus APP mice with higher Aβ levels exhibit greater disruption in autoregulation. This effect occurs in the absence of Aβ deposition in the brain and blood vessels. The data indicate that Aβ induces an alteration in the relationship between cerebral perfusion pressure and CBF that renders the brain more vulnerable to the deleterious effects of arterial hypertension and hypotension. This is a previously unrecognized biological effect of Aβ that has critical implications for the structural and functional integrity of the brain.

The disruption of autoregulation cannot be the result of alterations in body temperature or arterial blood gases, because these variables were monitored and carefully controlled. Certain anesthetics such as halothane abolish autoregulation (34). To avoid this problem, we used urethane-chloralose anesthesia, and autoregulation was present in nontransgenic mice. Therefore, anesthesia-related effects are not involved in the dysfunction of autoregulation. In addition, the disruption of the upper limit of autoregulation cannot be the result of pharmacological effects of the agent used to elevate MAP, because phenylephrine does not have confounding cerebrovascular effects (24), and for this reason it is often used to study the effects of hypertension on CBF (e.g., Ref. 8). It is unlikely that the cerebrovascular abnormalities in APP mice are the nonspecific result of varying degrees of global brain dysfunction for several reasons. First, although cerebrovascular autoregulation, functional hyperemia (31), and endothelium-dependent responses are altered in Tg6209, Tg2123M, and Tg2123F mice, responses to hypercapnia, hypocapnia, and to the NO-donor SNAP are preserved. Therefore, these mice do not exhibit failure of all cerebrovascular responses, which is what might occur if the alterations reflected global brain dysfunction. Second, in Tg2123M mice, which have higher levels of brain Aβ than Tg6209 and Tg2123F mice, the disruption in autoregulation is not associated with alterations in brain energy metabolism as was assessed by the 2-deoxyglucose method (29). Third, Tg6209, Tg2123F, and Tg2123M are lines that do not develop amyloid deposition in brain and blood vessels. Although Tg2576 mice develop amyloid plaques and other neuropathological abnormalities, we studied them at an age (2–3 mo) when these alterations are not present (15). Therefore, the cerebrovascular dysfunction cannot be attributed to gross neuropathological abnormalities. Fourth, the selective alterations in endothelium-dependent relaxation and functional hyperemia observed in APP mice can be reproduced in normal mice by topical application of synthetic Aβ (27, 30). Collectively, these observations indicate that the disruption in autoregulation that is observed in APP mice is not related to flaws in the experimental preparation or to factors that affect cerebrovascular reactivity nonspecifically.

To maintain flow in the autoregulated range of MAP, cerebral resistance vessels undergo vasoconstriction during hypertension and vasodilatation during hypotension (7). Therefore, failure of vasoconstriction and vasodilation may result in disruption of autoregulation. To determine whether such a mechanism was involved in the alteration of autoregulation in APP mice, we investigated the reactivity of the cerebral circulation to agents that increase or decrease CBF. We found that, in agreement with previous observations (13), the increase in CBF produced by the endothelium-dependent vasodilators ACh, BK, and A-23187 are markedly attenuated in APP mice. A new finding, however, was that the effect is more pronounced in transgenic lines with higher Aβ levels. This new observation provides a link between brain Aβ concentration

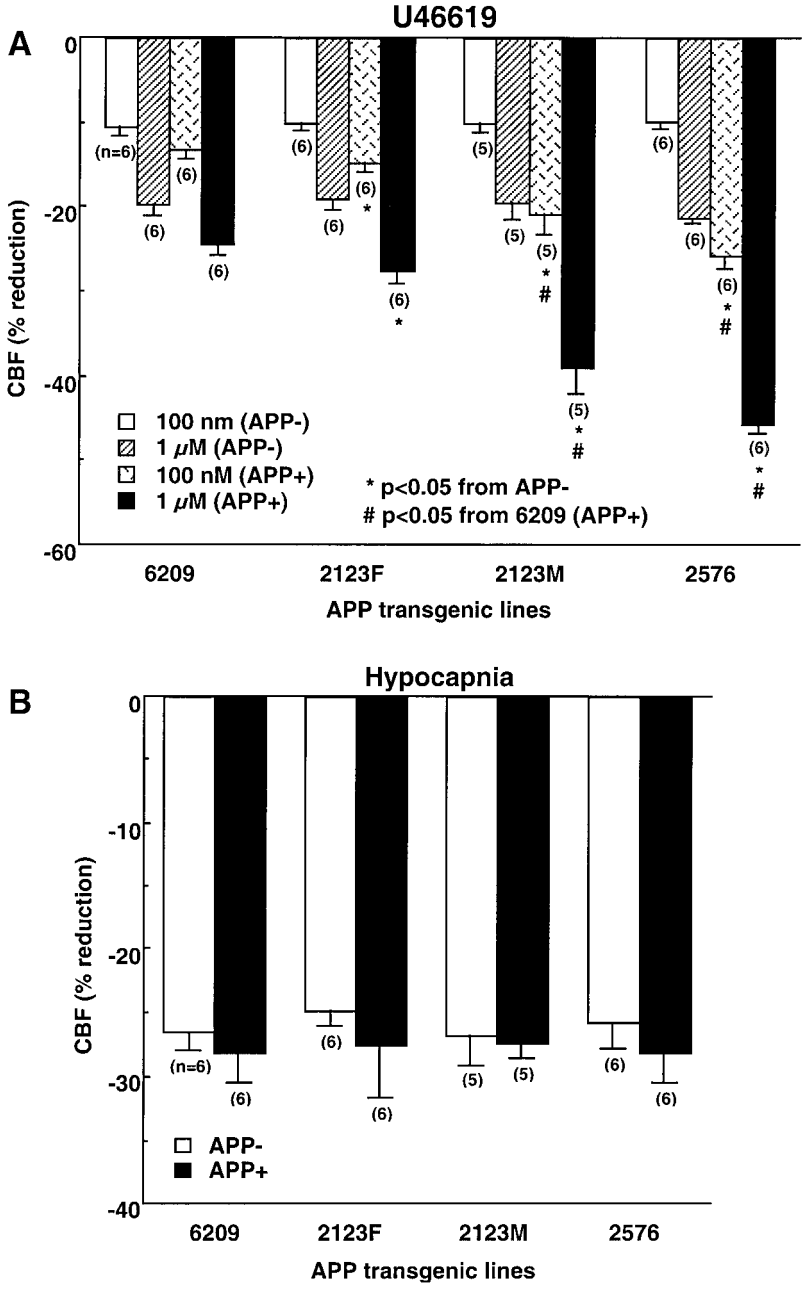

Fig. 6. Reduction in CBF produced by hypocapnia (*B*) and by the thromboxane analog U-46619 (*A*) in the different transgenic lines studied. $*P < 0.05$, *t*-test; $\#P < 0.05$, ANOVA and Tukey's test.

and disruption of endothelium-dependent vascular responses. The alteration in endothelium-dependent vasodilation could contribute to the loss of the lower limit of autoregulation by reducing the ability of the vessels to dilate when MAP is lowered. In support of this hypothesis, the endothelium is involved in the response of smooth muscle cells to pressure (6). Furthermore, inhibition of the synthesis of the endothelium-dependent vasodilator NO impairs the lower limit of autoregulation (20), and mice lacking endothelial NO synthase have altered autoregulation (11). However, we cannot rule out the participation of other factors as well. For example, ATP-activated potassium channels are involved in the vasodilation that occurs at low MAP (see Ref. 3 for a review) and may also play a role in the dysfunction that is observed in APP mice.

As for the upper range of autoregulation, a failure of vasoconstriction should be responsible for the alterations in CBF at high MAP. To determine whether there was a global impairment in vasoconstriction in APP mice, we tested the effect of interventions that decrease CBF. We found that the reduction in CBF produced by hypocapnia is not affected in APP mice, and that the CBF reduction produced by the thromboxane analog U-46619 is enhanced, an effect that is more marked in transgenic lines with higher levels of Aβ. Therefore, the failure of APP mice to autoregulate at high MAP cannot be attributed to a nonspecific defect of cerebral vessels to constrict. The vasoconstriction produced by hypertension is related to a "myogenic" constrictor response evoked by increased intravascular pressure (18). Although the loss of autoregulation dur-

ing hypertension could be related to an impairment of the myogenic response, this possibility needs to be tested experimentally.

We have previously demonstrated that the cerebrovascular alterations induced by Aβ are mediated by ROS. This conclusion is based on several pieces of evidence. First, the alterations of endothelium-dependent relaxation produced by synthetic Aβ applied to the cerebral cortex of normal mice or to isolated blood vessels are abrogated by ROS scavengers (27, 30, 38). Second, the alterations of endothelium-dependent relaxation in APP mice are counteracted by topical application of the ROS scavenger superoxide dismutase (13). Third, overexpression of Cu,Zn-SOD in APP mice prevents the cerebrovascular effects of Aβ (13). Fourth, substitution of methionine in position 35 with isoleucine, a mutation that prevents Aβ from producing ROS, eliminates the cerebrovascular effects of the peptide (27, 30). Our working hypothesis is that Aβ leads to vascular ROS production that is similar in cellular source and magnitude to that evoked by homocysteine, diabetes, or hypertension, which are conditions that produce ROS-mediated cerebrovascular alterations (3). Although there is no direct experimental evidence that ROS contribute to the alteration in autoregulation, the fact that the other vascular effects of Aβ are mediated by ROS strongly suggests that these agents are also involved in the impairment of autoregulation.

Another new finding of the present study is that in Tg2676 mice, which is the transgenic line in which Aβ levels and ADI are greatest, CBF responses to both endothelium-dependent and -independent vasodilators are attenuated. This observation suggests that in Tg2576, unlike the other lines, the loss of autoregulation at low MAP is due to a global impairment of cerebrovascular reactivity. The reasons for the greater impairment in cerebrovascular dilatation in Tg2576 are unknown. Amyloid deposition in blood vessels can be ruled out because mice were studied at 2–3 mo of age when brain Aβ levels are elevated but there is no amyloid deposition in the tissue (9). We doubt that structural alterations of the endothelium play a role, because endothelial cells do not exhibit ultrastructural abnormalities in APP mice (13). Therefore, one possibility is that the higher levels of Aβ attained in Tg2576 mice have direct effects on cerebrovascular smooth muscle cells impairing the ability to relax. Ongoing studies are addressing this issue.

The present findings provide an explanation for the observation that middle cerebral artery (MCA) occlusion produces more severe ischemia and larger infarcts in APP mice (41). Loss of autoregulation impairs the ability of cerebral resistance vessels to dilate in response to the reduction in transmural pressure produced by MCA occlusion and leads to more severe ischemia. In addition, it must be kept in mind that resting neocortical flow is reduced by 20–40% in APP mice (Tg2123 and Tg2576; Ref. 29). Therefore, absolute CBF values at low intravascular pressure are even lower than anticipated on the basis of the relative flow measurement by LDF. However, it is unlikely that the disruption in autoregulation in APP mice results from the reduction in resting CBF, because reductions in CBF of 50% do not affect autoregulation (2).

It has long been speculated that cerebrovascular dysfunction contributes to AD (see Ref. 21 for a review). Pathological studies of AD brains have reported rarefaction, thickening, and coiling of cerebral blood vessels and white-matter ischemic changes (4, 21), whereas studies using functional brain imaging have shown that AD patients have reduced CBF and glucose utilization (19). The response to hypercapnia is preserved in AD patients (26). However, the pathogenic significance of these cerebrovascular changes has remained unclear because the possibility that these changes were a consequence of the neuronal dysfunction, gliosis, and atrophy occurring in AD was not ruled out. The findings of the present study demonstrate that in APP mice, cerebrovascular dysfunction precedes the neuropathological alterations. Inasmuch as the pathology in APP mice reflects that of AD, the results support the view that the cerebrovascular effects of Aβ are an early event in AD and as such may play a pathogenic role in the mechanisms of the disease. Although to our knowledge the full range of cerebrovascular autoregulation has not been studied in AD, alterations in cerebrovascular autoregulation have important implications for the functional and structural integrity of the brain. Loss of autoregulation renders the brain more susceptible to fluctuations in MAP, such as those occurring during sleep (14). Thus reductions in MAP that would not alter cerebral perfusion in the normal brain may lead to cerebral ischemia in the presence of Aβ. Hypoperfusion-related ischemia would be most marked in the periventricular white matter, an area supplied by terminal arterioles with limited collateral flow (22, 25). These observations raise the possibility that an impairment in autoregulation contributes to the periventricular white-matter lesions that are frequently observed in patients with AD (1).

We conclude that overexpression of APP in mice produces a profound disruption in cerebrovascular autoregulation that is more pronounced in transgenic lines with high levels of brain Aβ. The Aβ-related failure of autoregulation is paralleled by a disruption of endothelium-dependent vasodilatation that is also greater in lines with high brain Aβ levels. Thus Aβ renders the brain more vulnerable to the variations in MAP that occur during normal activities. The data unveil a novel aspect of the vascular biology of Aβ and support the view that cerebrovascular alterations may play a role in the mechanisms of AD and related conditions.

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