Hypertension Induces Brain β-Amyloid Accumulation, Cognitive Impairment, and Memory Deterioration Through Activation of Receptor for Advanced Glycation End Products in Brain Vasculature

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Abstract—Although epidemiological data associate hypertension with a strong predisposition to develop Alzheimer disease, no mechanistic explanation exists so far. We developed a model of hypertension, obtained by transverse aortic constriction, leading to alterations typical of Alzheimer disease, such as amyloid plaques, neuroinflammation, blood-brain barrier dysfunction, and cognitive impairment, shown here for the first time. The aim of this work was to investigate the mechanisms involved in Alzheimer disease of hypertensive mice. We focused on receptor for advanced glycation end products (RAGE) that critically regulates Aβ transport at the blood-brain barrier and could be influenced by vascular factors. The hypertensive challenge had an early and sustained effect on RAGE upregulation in brain vessels of the cortex and hippocampus. Interestingly, RAGE inhibition protected from hypertension-induced Alzheimer pathology, as showed by rescue from cognitive impairment and parenchymal Aβ deposition. The increased RAGE expression in transverse aortic coarctation mice was induced by increased circulating advanced glycation end products and sustained by their later deposition in brain vessels. Interestingly, a daily treatment with an advanced glycation end product inhibitor or antioxidant prevented the development of Alzheimer traits. So far, Alzheimer pathology in experimental animal models has been recognized using only transgenic mice overexpressing amyloid precursor. This is the first study demonstrating that a chronic vascular insult can activate brain vascular RAGE, favoring parenchymal Aβ deposition and the onset of cognitive deterioration. Overall we demonstrate that RAGE activation in brain vessels is a crucial pathogenetic event in hypertension-induced Alzheimer disease, suggesting that inhibiting this target can limit the onset of vascular-related Alzheimer disease. (Hypertension. 2012;60:188-197.)

Key Words: hypertension ■ Alzheimer disease ■ receptor for advanced glycation end products ■ cognitive impairment ■ basic science

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hypothesized that hypertension should affect this complex system of brain vessels. A pathological feature of AD, underlying the cognitive impairment and dementia, is the accumulation of amyloid-β peptide (Aβ) in the brain, and increasing evidence points out a central role for Aβ transport across the BBB in determining central nervous system concentrations of Aβ, given the ability of peripheral Aβ to interact with the cerebral vasculature and influence its own deposition in brain. The BBB maintains the right balance of the intracerebral pool of Aβ with the one of the bloodstream. Actually, the structural composition of the BBB does not allow free exchanges of polar solutes, such as Aβ, between brain and blood or the contrary. However, many mechanisms contribute to the physiological entrance and efflux of Aβ in and out the brain. Specialized receptors at the BBB permit the shuttling of Aβ across the brain endothelium from the central nervous system into the bloodstream or vice versa.

Among these receptor systems, the receptor for advanced glycation end products (AGE; RAGE) dominates the BBB transport of Aβ into the brain. So far, RAGE activation has been associated with the development of diabetes mellitus, and, only more recently, it has been demonstrated that RAGE is activated in AD murine models in which the pathology starts in the nervous system, like the transgenic models. However, there is no definitive evidence of whether blood pressure challenge can activate RAGE in brain vessels, triggering and sustaining Aβ precipitation in the brain.

To elucidate this issue we have exploited a particular murine model of arterial hypertension, obtained by transverse aortic coarctation (TAC) and prone to develop AD-related brain pathology. We have demonstrated previously that the hemodynamic challenge imposed by TAC on the brain led to cerebral amyloid deposition as early as 4 weeks later, preceded by hypoperfusion and neuroinflammation. The derived brain injury was mainly localized in selected brain areas controlling cognitive functions, such as the cortex and hippocampus. We have also shown that TAC-induced hypertension increases the formation of soluble oligomers and intermediate amyloids, the most neurotoxic forms of Aβ. Interestingly, it has been demonstrated that the severity of the cognitive defect in AD correlates with levels of oligomers in the brain, more than the total Aβ burden.

Methods
For detailed description of the methods please refer to the online-only Data Supplement.

Animals and Surgery
All of the experiments were conducted in conformity with European Communities Council Directive No. 86/609/EEC. Eight- to 12-week-old C57Bl/6j and RAGE knockout (RO) male mice on a C57Bl/6j background were used for all of the other experiments. Animals were kept under a constant 12-hour light-dark cycle at a temperature of 22°C to 25°C. Standard chow and water were provided ad libitum.

Hypertension was induced by TAC, performed in anesthetized mice, between truncus anonymous and left carotid, with a 7.0 nylon suture ligature placed around the aorta. Sham mice were used as control.

Drug Treatment
The AGE inhibitor aminoguanidine hemisulfate, 50 mg/kg per day (Sigma), and the antioxidant Tiron, 1.2 g/kg per day (Sigma), were given in drinking water starting 3 days before the induction of hypertension by TAC. The RAGE inhibitor FPS-ZM1 was synthesized as described previously and given to mice via daily oral gavages at 1 mg/kg of body weight.

Behavioral Tests
Hippocampal and cortical functions were tested by the Morris water maze and novel object recognition (NOR) task, respectively.

Immunofluorescence, Histology, and Image Analysis
All of the stainings were performed on a 30-μm coronal brain fixed section, and images were acquired with a DM4000B Leica fluorescence/optical microscope (Leica Microsystems, Wetzlar, Germany).

Dissection of Brain Areas for RNA Extraction
Total RNA was extracted from hippocampi and cortices using TRIzol reagent (Invitrogen, Eugene, OR) according to the manufacturer’s instructions.

Reverse Transcription and Quantitative PCR
Total RNA was transcribed into cDNA using the RT-PCR SuperScript III kit (Invitrogen) according to the manufacturer’s instructions. Real-time PCR was performed with SYBR green PCR master mix, following the manufacturer’s instructions, using an ABI Prism 7500 Sequence Detection System (Applied Biosystems Inc, Foster City, CA).

Statistical Analysis
Data are presented as mean±SEM. Group means were evaluated by 1-way ANOVA and 2-way ANOVA for factorial design, as required by study design, followed by Tukey honestly significant difference test for the behavioral data and by Bonferroni post hoc test for all other analysis (GraphPad Prism Software 5).

Results
Hypertension Induces Late-Brain Aβ Deposition and Cognitive Impairment
We here confirm with Congo red (Figure 1A, upper panel) staining, anti-Aβ antibody (Figure 1A, middle panel), and Thioflavin-S (Figure 1A, lower panel) staining that TAC-induced hypertension caused cerebral amyloid deposition in the cortex (Figure 1A) and hippocampus (data not shown). More importantly, we here provide evidence for the first time that chronic hypertension also affected cognitive functions and led to behavioral alterations typical of early phases of the pathology (Figure 1B through 1E). In particular, because the hippocampus and cortex are the brain areas mostly affected by AD, we investigated the behavioral performance of mice subjected to TAC in both hippocampus- and cortex-dependent tasks, the Morris water maze and the NOR task. Sham and TAC mice showed no difference in the learning performance of the visual phase of the water maze task, indicating that unexpected drawbacks attributed to the manipulation do not interfere with the ability to solve the maze (data not shown). By contrast, a difference in spatial learning emerged in the 6 days of the acquisition phase, with TAC mice displaying significantly higher latencies in finding the hidden platform compared with Sham mice (Figure 1B). Such cognitive impairment shown by TAC mice was confirmed in
the probe phases. In both of these phases, a difference in the time spent in the 4 quadrants between sham and TAC mice was found. TAC mice spent significantly less time than sham mice in the target quadrant, that is, the quadrant where the platform was located, indicating an impairment in the spatial memory domain (Figure 1C).

Data from NOR also confirmed the profile of cognitive deficit. Sham and TAC mice showed no difference in the objects exploration during the acquisition phase, indicating the same basal levels of exploration and object preference for all of the experimental groups (Figure 1D). During the retention phase, when one object was changed and a novel one was introduced in the arena, TAC mice did not show differences in exploration between familiar and novel objects, compared with Sham mice, suggesting a memory impairment for TAC mice in object recognition (Figure 1E).

So far, this animal model has the advantage of show a spontaneous evolution toward typical features of AD, starting from a hemodynamic challenge. Thus, we reasoned that it could enable us to dissect the molecular mechanisms that underlie vascular-related AD development.

RAGE Is Early Activated by Hypertension in Brain Vessels and Is Crucial for Aβ Deposition and Cognitive Impairment

Cerebral blood vessels constitute the first line of defense for the brain from a peripheral hemodynamic challenge, like high...
blood pressure. Interestingly, RAGE receptor is expressed on endothelial cells, and its expression can be modulated by several neurohumoral factors, as well as by the increase in the circulating levels of its ligands. In this regard, we found that the hypertensive challenge had an early and sustained effect of upregulation of RAGE expression in the cortex and hippocampus, as evidenced at mRNA (Figure 2A) and protein levels (Figures 2B and S1). More importantly, the double staining for RAGE and platelet endothelial cell adhesion molecule (PECAM) 1 clearly showed that RAGE was almost exclusively localized in brain vessels (Figure 2C).

To evaluate the mechanistic role of RAGE in hypertension-induced AD pathology, we performed the hemodynamic challenge induced by TAC on mice with genetic ablation of RAGE (RO mice). When we histologically evaluated brains of TAC RO and wild-type (WT) mice, with Thioflavin S, we found a clear reduction in the parenchymal Aβ deposits in TAC RO mice as compared with WT mice (Figure 3A). Strikingly, RO mice displayed a strong positivity for Thioflavin S staining confined to cerebral blood vessels. This result was confirmed by the colocalization of Thioflavin S (Figure S2A) and Aβ (Figure S2B) with platelet endothelial cell adhesion molecule 1 staining, raising the question of whether RAGE genetic ablation, by inhibiting the overall influx of Aβ in the brain, induced a redistribution in local accumulation of the deposits (eg, from parenchyma to blood vessels).

To look at whether the amyloid concentration in brain vessels found in TAC RO mice affected brain capillary function, we evaluated 2 markers of oxidative stress and inflammation. In particular, we found that the class B scavenger receptor CD36, which has been demonstrated to be involved in vascular oxidative stress and neurovascular dysfunction induced by Aβ,16 is markedly increased in TAC RAGE knockout mice as compared with TAC WT mice (Figure 3B).

However, interestingly, when we analyzed learning and memory abilities in RO mice, we found that, in the Morris water maze, TAC RO mice showed lower latency to reach the platform during acquisition phase (Figure 3C) and spent significantly more time than TAC WT in the target quadrant during the probe phase (Figure 3D). This result found support in data from NOR, in which TAC RO mice explored for a long time the novel object, and their performance became similar to that observed in sham mice (Figure 3E and 3F), indicating that, other than the amyloid concentration in brain capillaries, RAGE ablation protected mice from hypertension-induced learning and memory impairment.

Finally, to further strengthen the data obtained in the genetic model of RAGE ablation, we chronically treated WT mice with FPS-ZM1, a recently developed high-affinity tertiary amide RAGE-specific inhibitor that was shown to block Aβ binding specifically to the V-domain of RAGE and prevented Aβ40- and Aβ42-induced cellular stress in vitro and in vivo.15 In addition, FPS-ZM1 treatment has been shown to significantly reduce Aβ pathology, normalize cere-
bral blood flow responses, and improve cognitive performance in a transgenic model of AD.\textsuperscript{15} As expected, we found that FPS-ZM1 treatment elicited the same effects of rescuing amyloid deposition (Figure 4A) and cognitive impairment (Figure 4B and 4C) after TAC-induced hypertension, as seen in the conventional RAGE knockout model.

**High Blood Pressure Induces AD by Activating RAGE Through Oxidative Stress and Glycation Product Formation**

The increased expression of RAGE was associated with a peak in circulating AGEs (Figure 5A), as measured by ELISA in serum samples from TAC mice at various time points. On the other hand, the normalization of AGE levels observed later in TAC mice was accompanied with their accumulation in the vascular tissue, as shown by the positive staining for one of the main AGEs, carboxymethyl-lysine, that was even more marked in TAC RO mice (Figure 5B), indicating that ligand formation was independent on the presence of its receptor. To address whether the early increase in circulating AGE, the main RAGE ligand, could effectively be the trigger of the increased RAGE expression in brain vasculature, we treated mice with aminoguanidine, an inhibitor of AGEs.
formation, during the hypertensive challenge. A daily oral treatment with aminoguanidine prevented both the early and the sustained increase in RAGE expression, as indicated by the reduced mRNA levels in the cortex (Figure 5C) and hippocampus (Figure S3A), as well as the protein localized in brain vessels that was almost absent in treated mice (Figure 5E). More interestingly, the treatment rescued both the amyloid deposition (Figure 6A) and the impairment in hippocampal (Figure 6B and 6C) and cortical (Figure 6D and 6E) functions.

Finally, to address whether the AGE-induced activation of RAGE was driven by an oxidant stress-related mechanism, we chronically administered Tiron, an antioxidant agent, during TAC, finding not only that the increase of RAGE in brain vessels was prevented, as shown by mRNA levels in both cortex (Figure 5D) and hippocampus (Figure S3B) and double staining with platelet endothelial cell adhesion molecule 1 (Figure 5E), but also that mice were protected from amyloid deposition (Figure 6A) and cognitive impairment (Figure 6B through 6E). Overall, our results obtained with AGE inhibitor and antioxidant treatment, which do not affect D1-D2

Our results appear to have a double implication. On the one hand, we demonstrate here for the first time that chronic conditions of hypertension lead to deterioration of memory in the Morris water maze test that assesses hippocampal function and in the NOR test evaluating cortical function, thus closely resembling alterations typical of AD. Thus, together with our previous observations demonstrating that TAC-induced hypertension also reproduces other typical features of AD, such as brain amyloid deposition, hypoperfusion, and neuroinflammation, the data presented here showing memory impairment fully characterize this experimental condition as a model of vascular-induced AD. This aspect shows an unprecedented opportunity to have a spontaneous murine model of AD that is not based on transgenic overexpression of mutant proteins related to amyloid production. Indeed, genetic models of AD greatly advanced knowledge about pathogenic processes linked to the disease, but the cause of sporadic forms of AD, affecting the majority of patients, still remains undiscovered, highlighting the need for novel approaches to identify the molecular targets underlying this disease.

On the other hand, we have found the molecular switch of RAGE signaling, mediating transcytosis of plasma-derived Aβ across brain endothelium and inducing the AD-related pathology observed in TAC mice. In this regard, we have demonstrated previously that passive immunotherapy, obtained by administration of anti-Aβ IgG, is able to rescue hypertensive brains from Aβ deposition and plaque formation, supporting the fact that transport of Aβ across the BBB contributes to the overall concentrations and consequent deposition of Aβ in the central nervous system in our model.}

Discussion
In this study, we show that RAGE is the molecular target activated by hypertension to induce cognitive deterioration and amyloid deposition, typical traits of AD. In particular, we have found that high blood pressure, through the induction of oxidative stress, determines the formation of circulating AGEs that, in turn, recruit RAGE activation in brain capillaries. Finally, we show that inhibition of RAGE or of the oxidant stress-related AGE/RAGE axis, is able to prevent both cognitive deterioration and amyloid deposition induced by the long-standing hemodynamic challenge to the brain, imposed by TAC.

Figure 4. Receptor for advanced glycation end products (RAGE) inhibition with FPS-ZM1, a high-affinity specific inhibitor, protects from (A) amyloid deposition and cognitive impairment shown by (B) acquisition learning and (C) target plat quadrant preference in probe Morris water maze (MWM) test (*P<0.05 vs sham vehicle and transverse aortic coarctation [TAC] RAGE inhibition groups; #P<0.05 vs other quadrants). B, ○, sham vehicle; □, sham RAGE inhibition; grey circle, TAC vehicle; ■, TAC RAGE inhibition. C, □, sham vehicle; light grey box, sham RAGE inhibition; dark grey box, TAC vehicle; ■, TAC RAGE inhibition.
Moreover, so far RAGE has been involved in transgenic models of AD where the trigger of the disease depends on increased \( A\beta \) production. Moreover, recent data associate cerebral hypoperfusion to RAGE activation and cognitive deficits. Intriguingly, brain hypoperfusion is also one of the main consequences of hypertension that continuously challenges cerebral vessels.

Here we show that mice with genetic ablation for RAGE or treated with FPS-ZM1, a recently established high-affinity RAGE-specific inhibitor, are protected from hypertension-induced AD pathology. In particular, we show that, in our model, RAGE inhibition protects from transport of pathophysiologically relevant concentrations of \( A\beta \) into the central nervous system, hampering plaque deposition.

**Figure 5.** Hypertension induces (A) an early formation of circulating advanced glycation end products (AGEs) and (B) sustained deposition in brain vessels. C and D. Transverse aortic coarctation (TAC)–induced receptor for advanced glycation end products (RAGE) in brain vessels is prevented by a treatment with aminoguanidine (C) and Tiron (D), also shown by RAGE and platelet endothelial cell adhesion molecule (PECAM) 1 double staining; scale bar, 20 μm (E). *P<0.01 vs sham; #P<0.01 vs TAC vehicle.
formation, Aβ deposition around blood vessels, and cognitive impairment. Despite these beneficial effects, RAGE inhibition also determines the concentration of Aβ in brain capillaries, with consequent activation of oxidative stress and vascular inflammation.

With regard to how RAGE can be activated by high blood pressure, we looked at one of its main ligands, the AGE. It has been clearly demonstrated that AGEs can be increased by hyperglycemia and, in turn, produce an activation of RAGE on vascular endothelium, generating oxidative stress. On the other hand, recent observations put RAGE activation downstream the oxidative stress. In particular, it has been demonstrated that angiotensin II–induced activation of RAGE is impaired by using free-radical scavengers and that hyperglycemia-induced reactive oxygen species production increases expression of RAGE and RAGE ligands. Although an association among vascular stiffness and AGE formation has been described, and recent data report that AGEs induce AD-like pathology in rats, there are no definitive studies that correlate the hemodynamic stress to activation of the AGE/RAGE pathway. However, the development of vascular disease has its origins in an initial insult to the vessel wall by biological or mechanical factors. In particular, the increase in pressure-induced mechanical stress represents one of the main stimuli for reactive oxygen species generation in vessels, and the oxidative stress that follows can further recruit RAGE activation, as described for hyperglycemia-induced reactive oxygen species.

In our study we identify, for the first time, that high blood pressure can activate an AGE/RAGE pathway in the brain endothelium through oxidative stress. More importantly, we found that the inhibition of AGE formation and oxidative stress prevents the deposition of amyloid plaques and the development of cognitive impairment induced by hypertension. These findings have potential clinical impact, suggesting that vascular RAGE is a target for inhibiting pathogenic consequences of hypertension-induced Aβ-vascular interactions, neuroinflammation, development of cerebral amyloidosis, and cognitive impairment.
In conclusion, our data uncover for the first time that, in the current wide scenario-based genetically induced models of AD, a vascular hypertensive challenge recapitulates the main traits of AD pathology, thus fully supporting the epidemiological and molecular data obtained in humans. In this regard, in the last few years, the clinical attention from the classical “amyloid cascade” has been changed to a “dynamic polygon” view, where the vascular risk factors have the major impact. In this perspective, the results of the present study demonstrate that the vascular-induced AD pathology is mediated through a high blood pressure–induced RAGE mechanism, opening up a new therapeutic strategy in which switching off this molecule could allow us to cope with vascular-related AD.

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Disclosures

None.

References


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**Novelty and Significance**

**What Is New?**
- Hypertension and AD have been strongly associated in epidemiological studies. In this article we identify the molecular mechanism responsible for β-amyloid deposition and cognitive impairment in a hypertensive mouse model. The high translational potential of our finding is supported by the use of a novel drug inhibiting this pathway.

**What Is Relevant?**
- So far most studies have used engineered animal models mimicking genetic AD, a small fraction of AD cases. In contrast, using an animal model of hypertension-related AD, we identify for the first time that hypertension activates RAGE in brain vessels, leading to Aβ brain deposition and memory impairment.

**Summary**

Although the involvement of the RAGE in AD is already recognized, our observation, describing that hypertension activates a molecular cascade converging on brain vascular RAGE, opens up a new therapeutic strategy in which targeting this molecule could allow us to cope with vascular-related AD.