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;60:00.)

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

The central nervous system absorbs ≈20% of the whole cardiac output and relies on an elaborate vascular network not merely to supply nutrients but also to maintain neuronal homeostasis. Appropriate functioning of the cerebral circulation is crucial for preserving normal cognitive function. Aging and vascular factors are the dominating causes of cerebrovascular dysfunction and, arterial hypertension, the most widespread cardiovascular risk factor accompanying mid and late life, appears to be a main challenge for the onset and progression of dementia.

In particular, hypertension is a powerful risk factor for Alzheimer disease (AD), the most common cause of dementia in the elderly. Indeed, despite the old belief that AD is distinct from vascular dementia, having a nonvascular origin, a growing body of epidemiological studies strongly associate vascular risk factors, such as arterial hypertension, with increased probability to develop AD, reducing the boundary between AD and vascular dementia.

Many studies have focused on the possible mechanisms underlying the cognitive deterioration induced by hypertension, but a pathophysiological mechanistic link is still missing. Because brain vessels show unique structural and functional features, like the blood-brain barrier (BBB), allowing the fine crosstalk between vascular and nervous systems, we
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 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.

**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 DMI4000B 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.
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 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 (e.g., 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 perfor-
mance in a transgenic model of AD. 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

Figure 3. Receptor for advanced glycation end products (RAGE) ablation (RO mice) prevents development of hypertension-
induced (A) plaque formation in brain parenchyma, by shifting Aβ deposition in brain vessels (representative images of cortex are
presented; scale bar, 50 μm). B, CD36 activation is marked in brain capillaries of transverse aortic coarctation (TAC) RO mice, as
shown by double staining (representative images of cortex are presented; scale bar, 50 μm). C and D, RAGE ablation protects
from impairment in learning and memory abilities in the hippocampus-dependent Morris water maze (MWM) in both acquisition
learning (C) and in probe phases (D; *P<0.05 vs other experimental groups; #P<0.05 vs other quadrants), as well as prevents
(E and F) memory deficits as show by retention phase of novel object recognition (NOR; F; *P<0.05 vs familiar object). C, ○,
sham wild type (WT); grey circle, TAC WT; □, sham RO; △, TAC RO. D through F, □, sham WT; light grey box, TAC WT; dark
grey box, sham RO; ■, TAC RO.
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 S3B). 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 the reduced mRNA levels in the cortex (Figure 5C) and the sustained increase in RAGE expression, as indicated by 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 challenged by high blood pressure to induce the multifaceted aspects of AD. Starting from the consideration that RAGE has been found upregulated in human AD brains, has a pivotal role in brain Aβ deposition in AD experimental models, and has been proposed as a fundamental mechanism signaling danger to vulnerable vasculature, we hypothesized that high blood pressure might interfere with 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.
Moreover, so far RAGE has been involved in transgenic models of AD where the trigger of the disease depends on increased Aβ 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 Aβ into the central nervous system, hampering plaque formation.
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.22 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 scavengers23 and that hyperglycemia-induced reactive oxygen species production increases expression of RAGE and RAGE ligands.23 Although an association among vascular stiffness and AGE formation has been described,24,25 and recent data report that AGEs induce AD-like pathology in rats,26 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.27 In particular, the increase in pressure-induced mechanical stress represents one of the main stimuli for reactive oxygen species generation in vessels,28 and the oxidative stress that follows can further recruit RAGE activation, as described for hyperglycemia-induced reactive oxygen species.23

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.

Figure 6. A daily oral treatment with aminoguanidine and Tiron rescues from hypertension-induced Aβ deposition (A) and from cognitive impairment (B through E). Mice treated with aminoguanidine and Tiron show a behavioral performance similar to sham vehicle mice in both the acquisition and probe phases of Morris water maze (MWM; B and C; *P<0.05 vs all other groups; §P<0.05 transverse aortic coarctation (TAC) vehicle vs sham vehicle and TAC advanced glycation; £P<0.05 TAC Tiron vs sham vehicle and TAC advanced glycation groups; #P<0.05 vs other quadrants), as well as in memory retention of novel object recognition (NOR) test (E; *P<0.05 vs familiar object). B, ♣, sham vehicle; grey diamond, TAC advanced glycation; □, TAC vehicle; *, TAC Tiron. C through E, ◇, sham vehicle; light grey box, TAC advanced glycation; dark grey box, TAC vehicle; ■, TAC Tiron.
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.\(^2\)\(^3\) 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.\(^2\)\(^3\) 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.

**Perspectives**

Despite the established clinical link between hypertension and AD, only a few basic science studies have investigated the specific molecular relationship so far. In particular, most studies use engineered animal models that mimic genetic AD, constituting only a small fraction of AD cases. In contrast, we have pursued the strategy to develop the only animal model of hypertension-related AD pathology so far. In fact, mice that have been subjected to high blood pressure show accumulation of amyloid aggregates, the main histological finding of AD, and, for the first time here, impairment in learning and memory tasks. More importantly, we identify that a hypertensive challenge activates oxidative stress in cerebral vessels responsible for an increased expression of RAGE, leading to Aβ brain deposition and memory impairment. The involvement of the RAGE axis in AD pathology is well recognized, but our observation is the first one describing that a vascular challenge can activate an oxidative molecular cascade converging on RAGE activation in brain vessels leading to the onset of the main traits of AD pathology.

**Sources of Funding**

The work was supported by grants from Italian Ministry of Health “Ricerca Corrente 2009,” “Cinquè per Mille,” and Italian Society of Hypertension (to G.L.) and a grant from the National Institutes of Health (AG17490; to S.S.Y.).

**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.
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_Hypertension_. published online May 21, 2012;
_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/early/2012/05/21/HYPERTENSIONAHA.112.195511

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HYPERTENSION INDUCES BRAIN β-AMYLOID ACCUMULATION, COGNITIVE IMPAIRMENT AND MEMORY DETERIORATION THROUGH ACTIVATION OF RAGE IN BRAIN VASCULATURE.

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Short Title: Hypertension, cognitive deterioration and RAGE.

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Methods

Animals and surgery
TAC-induced hypertension was performed in mice anesthetized with ketamine/xylazine, between truncus anonymous and left carotid, with a 7.0 nylon suture ligature placed around the aorta, with the two ends of the suture left outside the chest. A further group of mice underwent the same surgical procedures without realizing aortic stenosis (sham). A systolic trans-stenotic gradient was measured by echo Doppler (Vevo2100, Visualsonics inc., Canada), positioning the probe on the aortic banding. Blood pressure was monitored by in right carotid artery, via cannulated high fidelity micromanometers (Millar Instruments).

Behavioral tests
Morris Water Maze
The water-maze apparatus consisted of a white Plexiglas circular pool 88 cm in diameter and 33 cm in height, filled with water kept at a temperature of 26±1 °C. A plastic transparent platform (8 cm in diameter) was placed 0.5 cm below the water surface and 10 cm from the edge of the pool. All tests were carried out between 9:00 hr and 14:00 hr. The entire procedure took eight days. Mice were individually transferred from the home-cage to pool. To avoid visual orientation prior to release, mice were transferred from their cages into the pool in a non-transparent plastic cup. Release points were balanced across 4 symmetrical positions on the pool perimeter. The ability of experimental subjects to identify and reach a visible platform was tested in one visual cued version of the task which preceded the 6-days of acquisition phase. Each day mice underwent 3 trials during which they were allowed to freely swim for 60 sec or until they found and climbed onto the platform; each trial was spaced from the other by a 40 minutes inter-trial interval. Platform finding was defined as staying for at least 3 sec on it. During the acquisition phase, mice that did not find the platform were trained in locating it by placing them on the platform for 10 sec at the end of the trial. On the fourth day of the acquisition phase and 24 hours after the last acquisition trial, the platform was removed from the pool and each mouse was tested for memory retention in a 30-s probe trial. During the probe trial platform was removed from the pool and the time spent in the target quadrant of the maze (where the platform was located) was scored as a reliable measure of memory retention 1 or 24 hrs after the last acquisition trial. The swim path of the mice was recorded by means of a computer-based video-tracking system Ethovision (Noldus, Wageningen, Netherlands). For the probe phases, the variables recorded were time spent in each quadrant, number of platform crossing, mean swimming speed and thigmotaxis.

Novel Object Recognition Task
Objects used in training and testing trials presented distinctive colors and shapes and consisted of pairs of identical cans, small glass bottles, or blocks made with plastic Lego toys. Objects were chosen based an on preliminary data showing that these objects were of equal interest to naïve C57B6/J wild-type mice (data not shown). The different objects and their positions were counterbalanced across experiments and behavioral trials, and all objects had a height of about 10 cm. The objects were washed with a 40% ethanol solution between trials. Exploration was defined as sniffing or touching the object with the nose and/or forepaws, sitting on the object was not considered exploration. Mice were allowed to explore the empty arena for 10 min in the first 2 days (habituation). Twenty-four hours after habituation, training was conducted by placing individual rats into the field, in which two identical objects were positioned in two adjacent corners, 10 cm from the walls. Animals were left to explore the objects during 10 min and the time exploring each object was recorded. On memory retention test trials given 1 hr after training, mice explored the open field for 10 min in the presence of one familiar and one novel object.

Immunohistochemistry, histology and image analysis
In each experiment 5 mice for group were used for immunohistochemistry and histochemistry. Mice received an overdose of sodium pentobarbital and were transcardially perfused with 20 ml of
saline followed by 50 ml of ice-cold formaldehyde solution (4% paraformaldehyde in 0.1 M phosphate-buffered saline pH 7.4). Brains were removed, post-fixed overnight in the same fixative, and then placed in 20% sucrose/0.1 M phosphate buffer for at least 24 hrs. Coronal sections (20 μm) were cut on a freezing microtome and stored in cryoprotective solution at -20 °C. The following primary antibodies were used: anti RAGE (1:200, Santa Cruz); anti PECAM-1 (1:50, BD Biosciences Pharmingen); rabbit anti-β-Amyloid (1:100, Santa Cruz); anti-CML (CMS-10; 1:25, Abnova); anti-mouse CD36 (1: 50; Abcam). DAPI (Prolong Gold, Invitrogen, Molecular-Probes, Eugene, OR) was used for nuclear staining where indicated. For stainings with Congo Red and Thioflavin-S, brain sections were mounted on slides and allowed to air-dry over night and processed as described before. Images were captured using a DMI4000B Leica fluorescence/optical microscope (Leica Microsystems, Wetzlar, Germany) and processed with the Leica Application Suite (LAS V3.3). Since both cortex and hippocampus showed overlapping results, a representative image of one of two regions has been given, as detailed in figure legend. Dissection of brain areas for RNA extraction
Six mice per group were used for all quantitative PCR analysis. After decapitation, brains were immediately removed from the skull and transferred into ice-cold saline. Hippocampi and cortices were immediately dissected out, placed in plastic tubes, weighed, frozen on dry ice and stored at -80°C until RNA extraction. Total RNA was extracted using TRizol reagent (Invitrogen, Eugene, OR) according to the manufacturer’s instructions. Reverse transcription and quantitative PCR
Total RNA (1 μg) from each sample was transcribed into cDNA using the RT-PCR Superscript III kit (Invitrogen, Eugene, OR) 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). Expression levels of genes of interest were determined using the Relative Quantification (-ΔΔCt) Study of Applied Biosystems 7500 System SDS Software. Western Blotting
Brain proteins were extracted with lysis buffer (25 mM Tris, 150 mM NaCl, 1 mM each of NaF, Na3VO4, EDTA and Sigma protease inhibitor cocktail), centrifuged at 14,000xg for 30 min, and resolved by SDS-PAGE followed by Western blotting using anti-RAGE (1:200, Santa Cruz) and anti-GAPDH (1:5000, Santa Cruz) antibodies. Secondary antibodies used were: donkey anti-rabbit (1:5000) and sheep anti-mouse (1:2500), from GE Healthcare. Protein detection was performed with ECL kit (Amersham) and densitometry was obtained with the NIH Image 1.61 software. Serum AGEs
Circulating levels of AGEs were measured in serum samples by Cell Biolabs’ Oxiselect AGE ELISA kit.
Figure S1. TAC-induced hypertension early up-regulates protein levels of RAGE in hippocampus and cortex, as shown by western blot (representative images of n=4 for each group are presented with relative quantification; *p<0.05).
Figure S2. RAGE ablation (RO mice) prevents plaque formation in brain parenchyma, by shifting Aβ deposition in brain vessels, as shown by co-localization of Thioflavin-S (A) and anti-Aβ (B) with PECAM-1 (representative images of cortex are presented, scale bar 50 μm).
Figure S3. TAC-induced RAGE in hippocampus is prevented by a treatment with Aminoguanidine (A) and Tiron (B). *p<0.01 vs. sham and #p<0.01 vs. TAC-veh.
Figure S4. The chronic treatments with Aminoguanidine and Tiron do not affect blood pressure levels measured in carotid arteries (*p<0.001 vs Sham), thus indicating that they do not modify the hemodynamic challenge induced by TAC.