Published in final edited form as:

Cell Mol Neurobiol. 2009 September ; 29(6-7): 781–792. doi:10.1007/s10571-009-9368-4.

ANTI-INFLAMMATORY EFFECTS OF ANGIOTENSIN RECEPTOR BLOCKERS IN THE BRAIN AND THE PERIPHERY

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SUMMARY

In addition to regulating blood pressure, Angiotensin II exerts powerful pro-inflammatory effects in hypertension through stimulation of its AT₁ receptors, most clearly demonstrated in peripheral arteries and in the cerebral vasculature. Administration of Angiotensin II receptor blockers (ARBs) decreases hypertension-related vascular inflammation in peripheral organs. In rodent models of genetic hypertension, ARBs reverse the inflammation in the cerebral microcirculation.

We hypothesized that ARBs could be effective in inflammatory conditions beyond hypertension. Our more recent studies, summarized here, indicate that this is indeed the case.

We used the model of systemic administration of the bacterial endotoxin lipopolysaccharide (LPS). LPS produces a robust initial inflammatory reaction, the innate immune response, in peripheral organs and in the brain. Pretreatment with the ARB candesartan significantly diminishes the response to LPS, including reduction of pro-inflammatory cytokine release to the general circulation and decreased production and release of the pro-inflammatory adrenal hormone aldosterone. In addition, the ARB very significantly decreased the LPS-induced gene expression of pro-inflammatory cytokines and microglia activation in the brain. Our results demonstrate that AT₁ receptor activity is essential for the unrestricted development of full-scale innate immune response in the periphery and in the brain. ARBs, due to their immune response-limiting properties, may be considered as therapeutically useful in a number of inflammatory diseases of the peripheral organs and the brain.

Introduction

The present review summarizes our current studies on the role of Angiotensin II (Ang II), and in particular the participation of Ang II AT₁ receptors, in peripheral and brain inflammation. This work is a continuation of earlier findings demonstrating anti-inflammatory effects of Ang II AT₁ receptor blockers (ARBs) on the cerebral vasculature of Spontaneously Hypertensive Rats (SHR). We now demonstrate that: a) AT₁ receptor activity is essential for the development of a complete initial response to infection, the innate immune response; b) ARBs are potent anti-inflammatory compounds not only in peripheral organs but also in the brain; and c) the

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Abbreviations: Ang II – Angiotensin II; AT₁ – Angiotensin II receptor type 1; COX-2 – cyclooxygenase 2; ICAM-1 – intercellular adhesion molecule 1; IκBα – nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; IL-1β – interleukin 1β; IL-6 – interleukin 6; iNOS – inducible nitric oxide synthase; LBP – LPS binding protein; MAPKs – mitogen activated protein kinases; MCP-1 – monocyte chemoattractant protein-1; NFκB – nuclear factor κB; PGE₂ – prostaglandin E₂; PLA₂ – phospholipase A₂; ROS – reactive oxygen species; sCD14 – soluble CD14; TLR4 – Toll like receptor 4; TNF-α – tumor necrosis factor alpha; VCAM-1 – vascular cell adhesion molecule 1.

anti-inflammatory effects of ARBs occur beyond the anti-hypertensive effect of these compounds.

Angiotensin II is a major peripheral pro-inflammatory factor in hypertension, and ARBs reduce arterial inflammation in this disease

In addition to its role in the regulation of blood pressure and fluid metabolism, Ang II is now recognized as a pleiotropic factor involved in the regulation of multiple systems both in peripheral organs and in the brain (Saavedra et al. 2006b). Of prime importance is the pro-inflammatory effect of Ang II in the peripheral vasculature in hypertension (Cheng et al. 2005; Sanz-Rosa et al. 2005; Suzuki et al. 2003), because inflammation is now recognized as a major factor in the development and maintenance of this disease (Savoia and Schiffrin 2006; 2007). In hypertension, Ang II stimulates AT₁ receptors resulting in enhanced production and release of multiple pro-inflammatory factors, among those pro-inflammatory cytokines and aldosterone (Savoia and Schiffrin 2006). It has also been shown that in addition to reducing vasoconstriction, ARBs exert powerful anti-inflammatory effects in the peripheral vasculature (Marchesi et al. 2008).

Cerebrovascular Angiotensin II promotes vascular remodeling and enhanced vulnerability to brain ischemia. Both remodeling and vulnerability to ischemia are reversed by prolonged treatment with ARBs

Our laboratory has been focused on the role of Ang II in the control of the cerebral circulation (Saavedra and Nishimura 1999). We studied a rodent model of genetic hypertension, the SHR, exhibiting enhanced cerebrovascular Ang II AT₁ receptor expression (Zhou et al. 2006). In this model, we found that sustained administration of ARBs with access to the brain (Nishimura et al. 2000a) reversed the characteristic cerebrovascular remodeling (Nishimura et al. 2000b). As a result of this effect, ARBs protected the brain of SHR from the consequences of experimental stroke, substantially reducing the extent of ischemic damage by preserving the blood flow to the peripheral zone of penumbra (Ito et al. 2002; Saavedra et al. 2006c). Protection against ischemia by reversal of remodeling improves cerebrovascular compliance, and is considered an important mechanism explaining the proposed protection from stroke and the preservation of cognition characteristic of ARBs in human hypertension (Basile and Chrysant 2006; Bruce et al. 2008; Dahlöf 2006; Shlyakhto 2007).

Cerebrovascular inflammation participates in the vulnerability to brain ischemia in hypertension. ARBs reduce inflammation in the brain circulation of SHRs

We hypothesized that the protection of brain circulation offered by ARBs was not only the result of reversal of remodeling and preservation of the blood flow, but that central vascular anti-inflammatory mechanisms might also play important therapeutic roles. We asked the questions whether vascular inflammation in SHR extended to the cerebral vasculature and to what extent ARB administration might influence cerebrovascular inflammation in hypertension. We confirmed our hypothesis, demonstrating the presence of marked chronic inflammation in brain microvessels isolated from adult SHR and the capacity of ARB treatment to reverse the cerebrovascular inflammatory condition (Ando et al. 2004; Yamakawa et al. 2003; Zhou et al. 2005). The therapeutic anti-inflammatory effects of ARBs included reversal of cerebrovascular macrophage infiltration, normalization of the endothelial/inducible nitric oxide synthase (eNOS/iNOS) ratio, reversal of heat-shock protein upregulation, and reversal of the enhanced tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β) and inter-cellular

adhesion molecule 1 (ICAM-1) mRNA expression (Ando et al. 2004; Yamakawa et al. 2003; Zhou et al. 2005). In addition, ARBs reversed the stimulation of mRNA expression for the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) (Ando et al. 2004; Zhou et al. 2005), a transcription factor that plays a key role in regulating the immune response to infection.

The anti-inflammatory properties of ARBs are not restricted to their effects on the cerebral circulation of SHR. ARBs prevent stress-induced inflammation in the gastric mucosa

Ang II is a potent stress hormone, involved in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic system response to stress (Saavedra 2005; Saavedra and Benicky 2007). We discovered the anti-stress properties of ARBs when we found that blockade of AT₁ receptors by sustained peripheral administration of candesartan, a centrally-acting ARB (Nishimura et al. 2000a) prevented the sympathetic and hormonal response to isolation stress (Armando et al. 2001). We later found that blockade of AT₁ receptors prevented the cold-restraint stress-induced formation of gastric ulcers in the stress-prone SHR model (Bregonzio et al. 2003). In this model, acute ulcerations of the gastric mucosa are the consequence of a combined reduction of blood flow and a massive inflammatory reaction (Bregonzio et al. 2003). One of the principal mechanisms of the protective effect of ARBs was a major anti-inflammatory effect on the gastric mucosa. The ARB candesartan decreased the stress-induced gastric inflammation by reducing the upregulation of TNF- α and that of ICAM-1 in arterial endothelium, and by decreasing the neutrophil infiltration in the gastric mucosa (Bregonzio et al. 2003).

From the above we deduced that the anti-inflammatory effects of ARBs were not restricted to reversal of the chronic inflammation characteristic of the peripheral or brain circulation in hypertension. Instead, ARBs were also able to prevent the massive acute inflammatory reaction to cold-induced stress in the gastric mucosa. We hypothesized that ARBs may exert general anti-inflammatory effects not related to their role in blood pressure control. This hypothesis directed us to determine whether or not the anti-inflammatory effects of ARBs were restricted to hypertension or extended to other inflammatory conditions.

A model of peripheral and brain inflammation: the innate immune response to bacterial endotoxin

To clarify the role of AT₁ receptors, and that of ARBs, in inflammatory conditions unrelated to hypertension, we first asked the question to what extent treatment with ARBs influences the response to bacterial infection in normotensive animals.

A well-studied model of acute inflammation in rodents is the systemic administration of the bacterial endotoxin lipopolysaccharide (LPS), a major component of outer membranes of Gram-negative bacteria, followed by robust innate immune response in peripheral tissues and in the brain. After its recognition by soluble or membrane-bound CD14, LPS binds its signaling receptor, toll-like receptor 4 (TLR-4) (Triantafilou and Triantafilou 2005), expressed in multiple peripheral sites including macrophages located in the liver, spleen and other organs, and target sites in the brain microvasculature and circumventricular organs (Singh and Jiang 2004). This is followed by fast and transient release of pro-inflammatory cytokines to the circulation affecting all peripheral organs and the brain (Bosshart and Heinzelmann 2007; Han and Ulevitch 2005; Rivest 2003).

ARBs reduce the peripheral innate immune response to bacterial endotoxin

We first analyzed the effects of treatment with the ARB candesartan on the LPS-induced peripheral innate immune response. As a marker for peripheral inflammation, we determined the changes in circulating cytokines in normotensive rats pretreated with the ARB prior systemic administration of LPS. We found that the massive increase in plasma TNF- α and IL-6 produced by LPS was significantly reduced in animals pretreated with the ARB (Figure 1), as reported (Sanchez-Lemus et al. 2008). Earlier studies described a participation of AT₁ receptors in the LPS-induced production of pro-inflammatory cytokines in dehydrated animals (Miyoshi et al. 2003), probably the consequence of enhanced AT₁ receptor expression during reduced water intake (Sanvitto et al. 1997).

Our results indicate that: a) AT₁ receptor activation is necessary for the full development of the peripheral innate response in normal animals, suggesting this is a general phenomenon not related to particular physiological states; and b) the most likely sites for the action of the ARBs are tissue macrophages in organs such as the spleen and liver, expressing high levels of AT₁ receptors (Tsutsumi et al. 1992), and where most of the pro-inflammatory cytokines are formed (Dong et al. 2007; Wluka and Olszewski 2006). The conclusion is that ARBs, by blocking AT₁ receptor activation, limit the extent of the peripheral innate immune response.

In addition to acute inflammatory response, LPS activates the HPA axis, a classical hormonal response which includes enhanced hypothalamic corticotropin-releasing factor (CRF) formation and release, followed by increased adrenocorticotrophic releasing hormone (ACTH) release from the pituitary gland, leading to augmented release of anti-inflammatory glucocorticoids and pro-inflammatory mineralocorticoids from the adrenal gland (Grinevich et al. 2001; Moncek et al. 2003). Besides regulating the HPA axis activity, LPS acts directly on the adrenal gland to enhance cyclooxygenase-2 (COX-2) activity leading to the stimulation of local inflammatory processes and glucocorticoid release (Cover et al. 2001; Ichitani et al. 2001; Vakharia and Hinson 2005). The AT₁ receptor contributes to regulate glucocorticoid secretion from the adrenal gland through stimulation of hypothalamic CRF formation and release (Armando et al. 2007). Endogenously produced glucocorticoids are anti-inflammatory agents, protecting the brain and peripheral organs from the pathological consequences of an unrestricted innate immune response (Nadeau and Rivest 2003). AT₁ receptor signaling is also a major regulatory factor for the production and release of the adrenal mineralocorticoid aldosterone (Connell and Davies 2005). The aldosterone receptor, the mineralocorticoid receptor, is highly expressed not only in kidney but also in the brain (Gomez-Sanchez 2004), and chronic aldosterone secretion has been linked with brain inflammation, injury and stroke (Stier et al. 2005). For these reasons we focused on the effects of AT₁ receptor blockade on the LPS-induced formation and release of these adrenal hormones.

We found that systemic administration of LPS strongly activated the HPA axis leading to enhanced corticosterone and aldosterone release (Sanchez-Lemus et al. 2008). While pretreatment with the ARB did not affect circulating levels of corticosterone, it significantly reduced the initial phase of aldosterone release (Sanchez-Lemus et al. 2008) (Figure 2A). LPS-induced aldosterone release was accompanied by enhanced expression of aldosterone synthase mRNA and protein, and increased adrenal content of the hormone (Figure 2B) (Sanchez-Lemus et al. 2008). All these effects of LPS were completely prevented by pretreatment with the ARB (Figure 2B) (Sanchez-Lemus et al. 2008). In addition, the ARB reduced not only LPS-induced but also basal expression of aldosterone synthase mRNA (Figure 2B) (Sanchez-Lemus et al. 2008).

We concluded that: a) LPS exerts a direct effect on the adrenal gland, stimulating aldosterone transcription and release; b) the LPS effect on aldosterone synthase transcription and translation

has an absolute requirement for AT₁ receptor activation; c) AT₁ receptor function is essential for maximal aldosterone release; d) AT₁ receptor activity regulates the steady state of aldosterone synthase transcription in the adrenal gland under control conditions; e) the stimulating effect of LPS on ACTH and corticosterone release is unaffected by previous ARB treatment. The overall effect of ARB treatment is reduction of adrenal formation and release of a pro-inflammatory hormone, aldosterone, while preserving the release of the anti-inflammatory glucocorticoid, corticosterone.

The central innate immune response

The brain actively participates in the innate immune response (Hopkins 2007; Quan et al. 1997; Rivest 2003). This central component is activated very soon after LPS administration, and the stimulation of participating brain structures is dependent on effects of circulating cytokines and direct access of LPS to the brain (Quan and Banks 2007; Rivest 2003). In addition to fever induction and increased production of pro-inflammatory factors in the brain, administration of LPS induces a clear stress response with massive HPA axis stimulation due to activation of the hypothalamic paraventricular nucleus (PVN), another evidence of a close linkage between the hormonal and immune systems (Quan and Banks 2007).

The exact routes of communication between the brain and the immune system have been extensively studied but are not yet completely understood (Konsman et al. 2002; Quan and Banks 2007; Rivest 2003; Singh and Jiang 2004). They include direct neural information, signaling through the circumventricular organs and the endothelial cells of the blood-brain barrier (Dauphinee and Karsan 2006), transport of circulating cytokines to the brain, and local secretion from endothelial cells (Quan 2008; Quan and Banks 2007; Singh and Jiang 2004).

ARBs reduce the brain innate immune response to bacterial endotoxin

The initial purpose of our studies was not to clarify the mechanisms of brain-immune interactions, but simply to determine to what extent AT₁ receptors participate in the brain response to LPS, and whether or not AT₁ receptor inhibition modify the response to the endotoxin. We were encouraged in our studies by the early findings of large numbers of AT₁ receptors in all circumventricular organs and the PVN (Tsutsumi and Saavedra 1991a, 1991b), by the report of an existence of a local renin-angiotensin system in the cerebral microcirculation (Zhou et al. 2006) and by the findings of clear anti-inflammatory properties of ARBs in the cerebral microvessels of hypertensive animals (Ando et al. 2004; Zhou et al. 2005).

We have previously reported that sustained treatment with the peripherally administered ARB candesartan, not only reversed the cerebrovascular inflammation in SHR, but also prevented the stress-induced HPA axis activation and the central sympathetic stimulation (Armando et al. 2001; Bregonzio et al. 2008; Saavedra et al. 2006a). These effects were interpreted as the result of blockade of brain AT₁ receptors by sustained ARB administration (Nishimura et al. 2000a). In particular, the anti-stress effects of the ARB were linked to AT₁ receptor inhibition in the PVN, the site of CRF formation (Armando et al. 2001; 2007), and one of the important areas linking the response to stress and inflammation (Quan et al. 2003; Rivest et al. 2000).

We initiated our studies with the determination of the effects of systemic LPS administration in the brain cortex. We reproduced the well-characterized and widespread central inflammatory response to LPS (Qin et al. 2007; Quan et al. 1997; 1999; 2003; Singh and Jiang 2004), demonstrating a major increase in the gene expression of multiple pro-inflammatory factors including TNF- α , IL-1 β , iNOS, ICAM-1 and vascular cell adhesion molecule 1 (VCAM-1) (Figure 3). Additionally, LPS enhanced expression of the inhibitory factor κ B- α , (I κ B- α) mRNA (Figure 3). Induction of I κ B- α mRNA parallels NF- κ B activation, involved in the

coordinated regulation of the immune response (Miyamoto and Verma 1995; Miyazaki et al. 2007).

We completed our experiments with an examination of the effects of LPS and ARB pretreatment on brain microglia, the central resident immune cells (Streit et al. 1999) and a major target for LPS in the brain (Lehnardt et al. 2003). In agreement with previous reports (Garden and Moller 2006; Henry et al. 2008), we found significant microglia activation by LPS in the brain cortex (Figure 4).

Pre-treatment with the ARB candesartan completely abolished the LPS-induced overexpression of TNF- α mRNA (Figure 3), reduced that of IL-1 β , iNOS, ICAM-1, VCAM-1 and IkB α (Figure 3), and significantly decreased the LPS-induced microglia activation (Figure 4). Our results obtained *in vivo* are in agreement with a recent report of inhibition of LPS-induced IL-1 β release and of morphological changes in cultured microglia by AT₁ receptor inhibition with the ARB losartan (Miyoshi et al. 2008).

The conclusions from these experiments are: a) systemic administration of LPS produces a widespread and profound innate immune response in the brain, with generalized increase in transcription of multiple pro-inflammatory markers and extensive microglial activation; b) both enhanced gene expression and activation of microglia are, to a significant degree, dependent on full AT₁ receptor function; c) AT₁ receptors participate in the complex recognition and activation pathways elicited by the bacterial endotoxin and circulating pro-inflammatory cytokines; d) the AT₁ receptor activity was necessary for a full innate immune response to bacterial endotoxin, not only in the periphery but also in the brain; e) ARBs, by significantly limiting and regulating the innate immune response, exert important central anti-inflammatory properties.

The mechanisms of the anti-inflammatory effects of ARBs are not completely understood. Part of the effects may result from limiting the peripheral pro-inflammatory cytokine overproduction and release, thus decreasing the cytokine-dependent activation of central pro-inflammatory factors. Blockade of AT₁ receptors in the circumventricular organs and in the cerebrovascular endothelial cells may limit not only the effects of circulating cytokines but also the direct effects of LPS in these structures (Ching et al. 2007; Quan and Banks 2007). AT₁ receptor blockade in the cerebrovascular endothelial cells may also limit the local production of pro-inflammatory cytokines and diffusible nitric oxide (NO) in cell components of the blood brain barrier. Since systemic administration of the ARB blocks AT₁ receptors in neurons behind the blood brain barrier, this may be an additional possible mechanism protecting neurons from immune system activation-mediated injury.

The mechanisms of microglia activation by LPS and the protection by ARBs appear to be indirect. In the brain, AT₁ receptors are expressed in neurons (Jöhren and Saavedra 1996), but not in microglia (unpublished results). On the other hand, microglia expresses the LPS receptor TLR-4 (Lehnardt et al. 2003). However, the passage of LPS through the blood brain barrier is very poor or non-existent (Singh and Jiang 2004), and the endotoxin activates the brain innate immune response through stimulation of LPS receptors located in the circumventricular organs (Laflamme and Rivest 2001) and in the cerebrovascular endothelial cells (Xia et al. 2006). Most of the evidence for the central anti-inflammatory effects of the ARBs points to a focal interaction among the LPS, cytokine, aldosterone and Ang II AT₁ receptors pathways (Konsman et al. 2002; Lemarié et al. 2008; Mehta and Griendling 2007). The most likely site of interaction is located in endothelial cells forming part of the blood brain barrier (Quan et al. 2003). Figure 5 is a simplified diagram indicating common Ang II AT₁ receptor and LPS-related pathways in cerebrovascular endothelial cells. The figure illustrates receptor

localization and stimulation of common signaling cascades leading to the production of pro-inflammatory factors, transcription factor activation and the resulting inflammatory response.

CONCLUSIONS

Pretreatment with the ARB candesartan considerably decreases the peripheral and brain innate response to acute injection of bacterial endotoxin in normotensive rats. These anti-inflammatory effects include: a) blockade of increased formation and release of pro-inflammatory cytokines; b) decreased production and release of the pro-inflammatory hormone aldosterone; c) preservation of the anti-inflammatory glucocorticoid release; and d) a major decrease in pro-inflammatory gene expression and microglia activation in the brain.

Our results demonstrate that pretreatment with an ARB clearly limits the innate immune response to endotoxin administration not only in the periphery but also in the brain. The effects of ARBs in the brain may result from inhibition of Ang II and LPS common signaling cascades resulting in decreased production of inflammatory mediators such as NO, prostaglandin E₂ (PGE₂) and reactive oxygen species (ROS) (Figure 5). The conclusion is that ARBs may be considered peripheral and central anti-inflammatory agents not only in hypertension but also in other inflammatory disorders.

The possible anti-inflammatory effects of ARBs, and in particular their effects in the brain, if confirmed, may have major therapeutic implications. Chronic brain inflammation has been linked to the development of a number of neuro-psychiatric diseases including depression, Parkinson's and Alzheimer's diseases (Dutta et al. 2008; Fassbender et al. 2004; Rogers et al. 2007). There are no effective central anti-inflammatory compounds devoid of major side effects. The ARB limited but did not eliminate the brain innate immune response. This is a property of interest, since the innate immune response is necessary to maintain and restore homeostasis, and pathological conditions may be the consequence of unrestricted inflammation. For these reasons the use of ARBs for the treatment of acute and chronic anti-inflammatory disorders of the brain may be very advantageous and warrants further investigation.

Acknowledgements

This research was supported by the Division of Intramural Research Programs, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, USA.

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Figure 1. Candesartan decreases LPS-induced pro-inflammatory cytokine release to the general circulation

Normal adult male Wistar Hanover rats have undetectable levels of the pro-inflammatory cytokines TNF- α and IL-6 in the circulation. Control rats received vehicle and/or sterile saline. LPS administration (50 μ g/Kg, intraperitoneally) induces massive cytokine release to the general circulation, as determined by ELISA three hours after endotoxin injection. Subcutaneous treatment with candesartan (1 mg/Kg/day for three days) does not affect cytokine release in control rats, but significantly reduces the pro-inflammatory cytokine release produced by LPS. Values are mean $\pm$ SEM for groups of seven to nine animals measured individually. * $P < 0.05$ compared with vehicle-saline treated group; # $P < 0.05$ compared with vehicle-LPS treated group; one-way ANOVA and *post hoc* Tukey's test. ND, Non detectable. Modified from Sanchez-Lemus et al. 2008.

Aldosterone release

Area Under Curve

Aldosterone synthase mRNA

Aldosterone synthase protein

Adrenal aldosterone content

Aldosterone synthase

β-actin

Vehicle

Candesartan

Figure 2. Candesartan decreases LPS-induced aldosterone release to the general circulation, adrenal aldosterone content and adrenal aldosterone synthase mRNA and protein expression

A. Effect of candesartan on aldosterone release. To study hormone release, rats were cannulated in the tail artery and treated with vehicle or candesartan as described in Figure 1. Blood samples were collected just before LPS injection and at the indicated times in conscious and undisturbed rats. Values represent mean $\pm$ SEM for groups of five to 10 animals measured individually. ^a $P < 0.05$ compared with correspondent vehicle-LPS treated group; two-way ANOVA and *post hoc* Bonferroni's test. Areas under the curve were measured from 0 to 2 hours, and from 2 to 4 hours. Note that candesartan significantly decreased the enhanced aldosterone release produced by LPS only when the area under the curve was estimated between 0 and 2 hours. Values are mean $\pm$ SEM for groups of seven to nine animals measured individually. * $P < 0.05$ compared with vehicle-saline treated group; # $P < 0.05$ compared with vehicle-LPS treated group; one-way ANOVA and *post hoc* Tukey's test.

B. Effect of candesartan on aldosterone synthesis. To study adrenal aldosterone content and aldosterone synthase mRNA and protein expression, rats were treated as described in Figure 1. Candesartan treatment completely prevented the LPS-induced increase in adrenal aldosterone content and aldosterone synthase mRNA and protein expression. Data for aldosterone synthase mRNA and protein correspond to relative levels after normalization to 18S rRNA for and β -actin, respectively. Note that candesartan treatment, in addition to its action to decrease LPS-induced effects on aldosterone formation and release, decreases the steady state expression of aldosterone synthase mRNA. Values are mean $\pm$ SEM for groups of seven to nine animals measured individually. * $P < 0.05$ compared with vehicle-saline treated group; # $P < 0.05$ compared with vehicle-LPS treated group; one-way ANOVA and *post hoc* Tukey's test. Modified from Sanchez-Lemus et al. 2008.

Figure 3. Candesartan significantly decreases LPS-induced TNF α , IL-1 β , I κ B α , iNOS, ICAM-1 and VCAM-1 mRNA expression in brain cortex

The rats were treated for 14 days with candesartan (1 mg/kg/day) or vehicle via subcutaneously implanted osmotic minipumps. At the end of the treatment the rats of each group were injected either with LPS (50 µg/kg i.p.) or saline and were sacrificed three hours after the injection. The expression of pro-inflammatory genes was detected in whole cortex extracts by quantitative real-time PCR. Expression of all genes was normalized to the level of 18s rRNA and shown as a percent of Vehicle-Saline group. Data are expressed as means $\pm$ SEM (n=5–8). * P<0.05 compared with Vehicle-Saline; # P<0.05 compared with Vehicle-LPS.; One-way ANOVA and Newman-Keuls *post hoc* test.

Figure 4. Candesartan suppresses LPS-induced microglia activation in the rat cingulate cortex
 The representative pictures of microglia staining and quantitative analysis of microglia activation in rat cingulate cortex. The rats were treated for 14 days with candesartan (1 mg/kg/day) or vehicle via subcutaneously implanted osmotic minipumps. At the end of the treatment the rats of each group were injected either with LPS (50 μ g/kg i.p.) or saline and were sacrificed three hours after the injection. The formaldehyde-fixed brains were cut into 16 μ m sections and microglia was stained with mouse monoclonal anti-rat Ox-42 antibody (CD11b/c, BD Pharmingen) and visualized with avidin-biotin enhanced horse radish peroxidase detection system. Microscopic images were acquired by AxioCam digital camera using AxioVision 4.6 software (Zeiss). For quantitative study, the images were subjected to color channel separation and particle analysis using Image J 1.37 software (NIH). Activated microglia was characterized by increased size, irregular shape and intensified Ox-42 staining. The Ox-42 positive area was used as a measure of microglia activation. Data are expressed as means $\pm$ SEM (n=5–8). * P<0.05 compared with Vehicle-Saline; # P<0.05 compared with Vehicle-LPS. One-way ANOVA and Newman-Keuls *post hoc* test.

Figure 5. Angiotensin II and LPS – induced signaling pathways in endothelial cells in the control of the innate immune response

Activation of AT₁ receptor signaling cascade leads to ROS, PGE₂ and NO production. Prostaglandins are very important effectors of the Ang II–induced vascular permeability. Stimulation of endothelial cells either with Ang II or with bacterial endotoxin LPS activates several common downstream signaling pathways, including activation of mitogen-activated protein kinases, predominantly JNK, p38 and ERK, and transcription factors NF-κB and AP-1, resulting in enhanced expression of inflammatory cytokines, chemokines, inducible enzymes and adhesion molecules mediating the interaction of endothelial cells with peripheral leukocytes. Pro-inflammatory cytokines activate microglia in brain parenchyma. Blockade of AT₁ receptors with candesartan eliminates Ang II – stimulated downstream signaling cascade, and suppresses activation of microglial cells. Another possible target in microglia regulation via candesartan could be AT₁ – expressing neuronal cells.