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REVIEW PAPER

Mineralocorticoid Receptors, Neuroinflammation and Hypertensive Encephalopathy

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Abstract

Worldwide, raised blood pressure is estimated to affect 35–40% of the adult population and is a main conditioning factor for cardiovascular diseases and stroke. Animal models of hypertension have provided great advances concerning the pathophysiology of human hypertension, as already shown for the deoxycorticosterone-salt treated rat, the Dahl-salt sensitive rat, the Zucker obese rat and the spontaneously hypertensive rat (SHR). SHR has been widely used to study abnormalities of the brain in chronic hypertension. This review summarises present and past evidence that in the SHR, hypertension causes hippocampal tissue damage which triggers a pro-inflammatory feedforward cascade affecting this vulnerable brain region. The cascade is driven by mineralocorticoid receptor (MR) activation responding to endogenous corticosterone rather than aldosterone. Increased MR expression is a generalised feature of the SHR which seems to support first the rise in blood pressure. Then oxidative stress caused by vasculopathy and hypoxia further increases MR activation in hippocampal neurons and glia cells, activates microglia activation and pro-inflammatory mediators, and down-regulates anti-inflammatory factors. In contrast to MR, involvement of the glucocorticoid receptor (GR) in SHR is less certain. GR showed normal expression levels and blockage with an antagonist failed to reduce blood pressure of SHR. The findings support the concept that MR:GR imbalance caused by vasculopathy causes a switch in MR function towards a proverbial "death" receptor.

Keywords Hypertension · Mineralocorticoid receptor · Microglia · Neuroinflammation

Hypertension and the Mineralocorticoid Receptor (MR)

High blood pressure (BP) shows an overall prevalence in 40% of adults aged 25 and older, and is a conditioning factor for cardiovascular diseases and stroke. Within the myriad of factors involved in the pathogenesis of hypertension, mineralocorticoids and the mineralocorticoid receptor (MR) play a role in about 15% of patients with essential hypertension (Martinez et al. 2009). The MR plays some role in primary hypertensive patients is also supported by pharmacological studies using the MR blockers spironolactone or eplerenone, which decrease both systolic and diastolic BP (Jeunemaitre et al. 1987; Tam et al. 2017). Hypertension research has greatly benefited from preclinical models, and in this regard, the spontaneously hypertensive rat (SHR), which reproduces several features of human essential hypertension, has become a valuable tool for understanding pathogenic factors involved in high blood pressure.

In this review, we summarise present and past evidence showing that high expression of MR and pro-inflammatory factors are at the forefront of hippocampal abnormalities found in SHR. The SHR phenotype include myelin loss, astrogliosis, oxidative stress, decreased neurogenesis, vascular remodelling, enhanced brain angiotensin II system, mitochondrial dysfunction, decreased nitric oxide synthase (NOS), neuronal atrophy, microgliosis and neuroinflammation (Sabbatini et al. 2002; Tomassoni et al. 2004; Ueno

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et al. 2004; Ando et al. 2004; Pietranera et al. 2006, 2010; Amenta et al. 2010; Benicky et al. 2011; Brocca et al. 2013; Avolio et al. 2018). In association with these pathological features, there is a role for the MR in SHR, because blockage of this receptor with a specific antagonist decreased BP whereas a GR antagonist was ineffective (Rahmouni et al. 2001). Concomitant to changes of steroid receptors, the hippocampus of SHR showed an imbalance of pro-inflammatory and anti-inflammatory factors, towards a prevalent pro-inflammatory profile. The susceptibility of the hippocampus to MR activation, inflammatory factors and hypertension, is in line with the selective vulnerability to neurotoxic factors shown by this limbic region (McEwen 1994). Thus, findings in SHR further support that adrenal steroids may turn from a protective and anti-inflammatory function into a neurotoxic, proinflammatory role under adverse circumstances (Busillo and Cidlowski 2013; Sorrells et al. 2014).

Functional Aspects of Central MR and GR

Steroid binding to brain MR and GR occurs in a region, time and concentration-dependent manner (Reul and de Kloet 1985; Ahima et al. 1991; Gomez-Sanchez 2014). MRs show a restricted expression compared to GRs, and localise preferentially in neurons of the hippocampal pyramidal layers and gyrus dentatus, and in extra-hippocampal neurons involved in control of BP, salt appetite and sympathetic drive. In organs such as the kidney, the enzyme 11beta-hydroxysteroid-dehydrogenase type 2 (HSD2) converts active glucocorticoids to inactive products, allowing MR to bind aldosterone and initiate transcription of factors involved in sodium reabsorption such as the epithelial sodium channel (ENac) and the Na,K-ATPase (Edwards et al. 1988a; Seckl 1997; Gomez-Sanchez 2014).

However, the MR of the hippocampus imposes an interesting paradox in receptor function, because it binds and responds to glucocorticoids rather than to the natural mineralocorticoid aldosterone. A responsible factor for this unusual behavior is the absence in hippocampus of the HSD2 isozyme that inactivates cortisol (in humans) and corticosterone (rats and mice). Instead, the hippocampus contains the HSD1 (11beta hydroxysteroid-dehydrogenase type I) isozyme that regenerates bio-active glucocorticoids and, therefore, amplifies their actions (Edwards et al. 1988b; Seckl 1997; Gomez-Sanchez 2014). Consequently, hippocampal MR is occupied most of the time by glucocorticoids, which levels in hippocampal cell nuclei measure 10–50 times higher than those of aldosterone (Yongue and Roy 1987). Due to this property, the MR of hippocampus is largely occupied by corticosterone, a phenomenon that contributes to the maintenance of the basal hypothalamic–pituitary–adrenal axis (HPA) tone.

During stress or at the peak of the circadian rhythm, glucocorticoids progressively occupy GRs to initiate the negative feedback mechanism that re-establishes basal HPA axis activity (Sapolsky et al. 1985; de Kloet 2014; Oster et al. 2017). In other brain regions, the MR exerts additional functions. For instance, in the paraventricular hypothalamic nucleus (PVN), MR-expressing sympathetic neurons project to the spinal cord and participate in blood pressure control (Chen et al. 2014). MR-immunopositive cells are also found in neurons regulating salt appetite, blood pressure control and certain types of behavior, notably the nucleus tractus solitarii (NTS) (Geerling et al. 2006; Geerling and Loewy 2006). Also the organum vasculosum of the lamina terminalis (OVLT), various circumventricular organs, the median preoptic nucleus (MnPO), amygdala and the bed nucleus of the stria terminalis (BNST) are MR-immunopositive (Sapolsky et al. 1985; Ahima et al. 1991). Because these tissues express the enzyme HSD2, the preferred ligand of MR in these tissues likely is aldosterone, as reviewed by Joëls and de Kloet (2017) and de Kloet and Joëls (2017).

In contrast to MR, GR shows a more generalised distribution to neurons and glial cells: astrocytes, microglia and oligodendrocytes (Bohn et al. 1991; McEwen 1994; Sierra et al. 2008). The low affinity GR binds natural and synthetic glucocorticoids but not aldosterone, and is found in hippocampus pyramidal cell layers CA1, CA2, and gyrus dentatus but present in very low levels in the CA3 pyramidal cells (Van Eekelen and De Kloet 1992). In the PVN, GR-expressing cells from parvocellular neurons exert a negative control of the ACTH secretagogues corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP). The GR involved in the negative feedback suppression of ACTH synthesis and release following stress is found in the ACTH-producing cells of the anterior pituitary. The GR in higher brain regions including the hippocampus promotes various aspects of behavioural adaptation, and hence, the stress-induced drive towards HPA axis activation (de Kloet et al. 2005).

Participation of Adrenal Steroids and Central MR in Experimental Models of Hypertension

The function and expression of central MR has been studied in models of hypertension other than SHR. The deoxycorticosterone acetate (DOCA) salt-treated rat has been traditionally employed to ascertain the role of adrenal steroids in hypertension and the control of salt appetite. In this regard, Janiak et al. (1990) have shown that icv administration of the MR antagonist RU 28318 attenuated the development of DOCA salt-induced hypertension, and Gomez-Sanchez et al. (1992) have demonstrated that RU 28318 also inhibited the hypertension produced by peripherally injected

aldosterone or DOCA in the Dahl salt-sensitive (SS) rat. In the same venue, Van den Berg et al. (1994) have used DOCA-implanted Wistar rats drinking 0.9% NaCl solution to show that blockade of MR with RU 28318 reduces sympathetic outflow and attenuates the systolic BP. Thus, the involvement of central MR in the DOCA-salt model is strongly supported by experimental data. Brain MR is also involved in the natriogenic effect of DOCA, a conditioning factor for hypertension, whereas the role of GR in this behaviour is inconclusive (Vallee et al. 1995). In the last work, we have shown that high doses of DOCA given to male rats produce a salt preference, and increases binding of (^{3}H)-corticosterone in whole hypothalamus, which contains regions responsible for natriogenesis.

The Dahl salt-sensitive (SS) rat model has also been used to discern the steroid and receptor involved in hypertension. Huang et al. (2009) have reported higher levels of aldosterone in hypothalamus of Dahl SS rats, and based on the hypotensive effect of an aldosterone synthase antagonist, postulated aldosterone as the hypertensinogenic MR ligand. Using qPCR for determination of aldosterone synthase, ELISA for steroid levels, and the aldosterone synthase inhibitor FAD286, Gomez-Sanchez et al. (2010) have concluded that aldosterone synthesis in brain of Dahl SS is higher than in Sprague–Dawley rats, although MR mRNA in several brain regions (brain stem, hypothalamus, hippocampus, cortex and cerebellum) measures lower in the latter strain.

More recently, it has been postulated that hypertension of the Dahl model followed a pathway involving the interaction of MR and angiotensin II receptors in the subfornical organ, with projections to the PVN and rostroventrolateral medulla (Wang et al. 2016). However, other authors have argued that aldosterone synthesis by the brain occurs in too little amounts, if any, to be physiopathologically relevant (Funder 2009; Oki et al. 2012). Thus, the biological role of neuroaldosterone in BP regulation of the Dahl SS rat needs further investigation.

Recently, Evans et al. (2016) have shown that mutant mice with conditional knockout of HSD2 in the NTS increase their salt appetite (Evans et al. 2016). Spironolactone could not entirely block the increased salt appetite, since an additional angiotensin II antagonist is needed. When offered saline, the mice develop hypertension, which is abolished after removal of saline as drinking solution. Moreover, the pressor effect to phenylephrine remains enhanced and the baroreflex is impaired in the mutants offered saline, suggesting an enhanced sympathetic drive, likely caused by additional corticosterone stimulation of the MR in the NTS after ablation of HSD2.

Hypertension is a major component of obesity and the metabolic syndrome. Obese subjects show 65–75% increased risk of high BP (Hall et al. 2015). There is some speculation supporting that obesity causes MR activation independent of aldosterone or angiotensin II (Hall et al. 2015). In the genetically obese Zucker rat, there is elevated plasma corticosterone, hyperactivity of the HPA axis in response to stress and decreased MR mRNA in all hippocampal regions (Mattson et al. 2004). The authors have speculated that increased HPA activity of the obese Zucker rat is due to lower levels of MR but not GR. Recent work has suggested that increased aldosterone synthesis plays a major role in obesity-related hypertension (Müller-Fielitz and Raasch 2013). Other authors have studied the diabetic obese Zucker rat; in this strain, they found increased MR mRNA levels in hippocampus and hypothalamus(Jöhren et al. 2007). In obese humans, treatment with the MR antagonist spironolactone improves hippocampal-dependent memory (Rotenstein et al. 2015). Therefore, there is variability regarding the status of the MR in obesity-related hypertension.

The Neuroendocrine System of Hypertensive Rats, with Emphasis in SHR

Adrenal steroids exert multiple effects in the brain, which have been the subject of several reviews (McEwen 1994; Gomez-Sanchez 2014; Joëls and de Kloet 2017). Under physiological conditions, mineralocorticoids regulate central cardiovascular responses, modulate ion fluxes and the sympathetic tone, interact with vasoactive neuropeptides, are involved in neurotransmission and neurogenesis, play a role in neuroendocrine feedback mechanisms and modulate memory and cognition (Ratka et al. 1989; Tanaka et al. 1997; Joëls and de Kloet 2017). While several of these effects contribute to maintenance of a normal brain function, high levels of circulating mineralocorticoids and/or excessive activation of MR produce undesirable effects, including the induction of salt appetite, damage to the vasculature, increased sympathetic drive, acceleration of brain aging, increased oxidative stress, neuroinflammation, decreased neurogenesis, increased susceptibility to ischemia and stroke and development and aggravation of hypertension, as demonstrated in several experimental models and in patients with essential hypertension (Sabbatini et al. 2002; Pietranera et al. 2006; Lopez-Campistrous et al. 2008; Huang et al. 2009; Martinez et al. 2009; Brocca et al. 2013; Gomez-Sanchez and Gomez-Sanchez 2014).

In 1960, Okamato and colleagues developed the SHR as a genetic model of human hypertension. Unexpectedly, this strain shows a dysfunctional neuroendocrine system. For instance, the presence of intact adrenals and corticosterone are essential for the development of hypertension in young SHR, which as adults show alterations of the corticosteroid negative feedback mechanism and the response to stress (Hashimoto et al. 1989; Gómez et al. 1996). As further evidence of neuroendocrine abnormalities, SHR

demonstrates a higher number of AVP mRNA and vasopressin V1aR-immunopositive cells in the magnocellular division of the PVN than WKY rats (Pietranera et al. 2004). In addition, MR activation appears to maintain hypertension in SHR, as shown by the blood pressure reducing effect of the MR antagonists RU28318 or spironolactone, whereas infusion of aldosterone increases blood pressure of adrenalectomized SHR, but not of the WKY normotensive rats (Kenyon et al. 1981; Rahmouni et al. 2001). In SHR maintained on the standard diet, the icv injection of RU28318 (10 or 100 ng) has no effect on cardiovascular and renal functions. However, the icv injection of 10 ng RU28318 in SHR after 3 weeks of high sodium intake (8% NaCl) causes a long-lasting decrease in systolic BP (Rahmouni et al. 2001). The presence of a dysfunctional MR in SHR is shown by increased binding capacity for this receptor in hippocampus and hypothalamus, and by enhanced activation, DNA binding and increased mRNA expression in the heart and peripheral microcirculation (Koch et al. 1982; Sutanto et al. 1992; Mirshahi et al. 1998; Konishi et al. 2003). Furthermore, the acetylation status of the MR changes its function in the heart of SHR, imposing an epigenetic modulation of receptor function (Kang et al. 2015).

The overactivation of MR in SHR accompanies other neurochemical alterations. For example, receptor overdrive and neuroinflammation may associate and aggravate the encephalopathy (Shen et al. 2015). Thus, the brain in hypertension resembles the cardiovascular system in hypertension, where MR becomes a proverbial "death receptor" (Funder 2004). The paradoxical activation of hippocampal MR by glucocorticoids signals a pro-phlogistic role of MR, more so if we deal with an overactivated receptor. As shown below, the serum and glucocorticoid-activated kinase1 (Sgk1), a marker of receptor functionality and involved in inflammation, was significantly elevated in SHR compared to Wistar Kyoto (WKY) rats. This finding supports an overactivated MR in SHR.

MR and GR Expression in WKY Rats and SHR

The neuroendocrine abnormalities of SHR include a dysfunctional regulation of adrenal steroid receptors in the brain. As far as 1968, Bruce McEwen found that exogenously administered corticosterone was preferentially retained by the hippocampus (McEwen et al. 1968); this early report was followed by the discovery of two receptor system in this brain, known as MR and GR (Reul and de Kloet 1985; Arriza et al. 1988). We have studied the expression of these receptors as a first approach to understand their role in the hippocampus of SHR. We employed 10 month-old male SHR and WKY rats with mean blood pressures > 180 mm Hg (SHR) or < 120 mm Hg (WKY rats). Male rats were used because of a gender difference in BP, which is lower in female SHR than in males (Reckelhoff 2001).

Striking strain differences were found by immunofluorescence microscopy regarding MR and GR protein expression in the dorsal hippocampus (Fig. 1a). The top image of Fig. 1a shows that MR (green fluorescence) distributed throughout pyramidal cell layers (CA1, CA2, CA3) and gyrus dentatus, whereas GR (red fluorescence) was present in the same regions with the exception of CA3. Visual comparison shows higher MR immunofluorescence staining and slightly higher GR staining in SHR vs. WKY rats (Fig. 1a). The merged images (yellow) showed enhanced co-localisation of both receptors in SHR hippocampus compared to WKY rats. This change is better appreciated in high power microscope images (Fig. 1a, lower image), which confirms abundant MR fluorescent cells in the CA1 region, with a higher co-localisation with the GR in the SHR compared to WKY rats. Results were also reproduced for the CA3 region, as reported by Brocca et al. (Brocca et al. 2017). Furthermore, quantitative analysis of dual immunofluorescence staining for MR/GR positive cells in the CA1 region of SHR demonstrated higher % of MR + cells with co-expression of GR (Fig. 1b). Likewise, a higher % of MR + cells co-localised with the GR protein in the CA3 region of SHR (Brocca et al. 2017). Figure 1c shows results of in situ hybridisation produced after exposing hippocampal sections to an oligonucleotide probe specific for MR (Pietranera et al. 2012). The film autoradiograms show intense probe localisation in SHR but a weaker mRNA signal for WKY rats in most hippocampal pyramidal layers and gyrus dentatus. MR gene transcription determined by qPCR has demonstrated that SHR express about 2.5-fold higher MR mRNA levels in whole hippocampus compared to WKY rats ($p < 0.01$), in agreement with a previous report (Pietranera et al. 2012). In similarity with transcriptional data, MR-immunoreactive protein in the CA1 and CA3 regions show larger total immunoreactive area / mm^2 in SHR compared to WKY rats, as reported by Brocca et al. (2017). In contrast, GR mRNA levels in whole hippocampus were similar in both strains. However, differences in GR total immunoreactive area were obtained in the CA1 region, favouring SHR over WKY rats. Thus, SHR are mainly distinguished from WKY rats by increased expression of MR mRNA and protein and enhanced co-localisation of MR/GR proteins in the hippocampus.

Measurement of steroid receptors was followed by determination of BP with and without the administration of receptor antagonists. Figure 2 shows the expected increased BP of SHR vs. WKY rats. To block the receptors, we have injected icv 400 ng of the specific MR antagonist RU28318 (Van den Berg et al. 1994) or the specific GR antagonist CORCEPT 113176 (Pineau et al. 2016) into anesthetized rats. Twelve

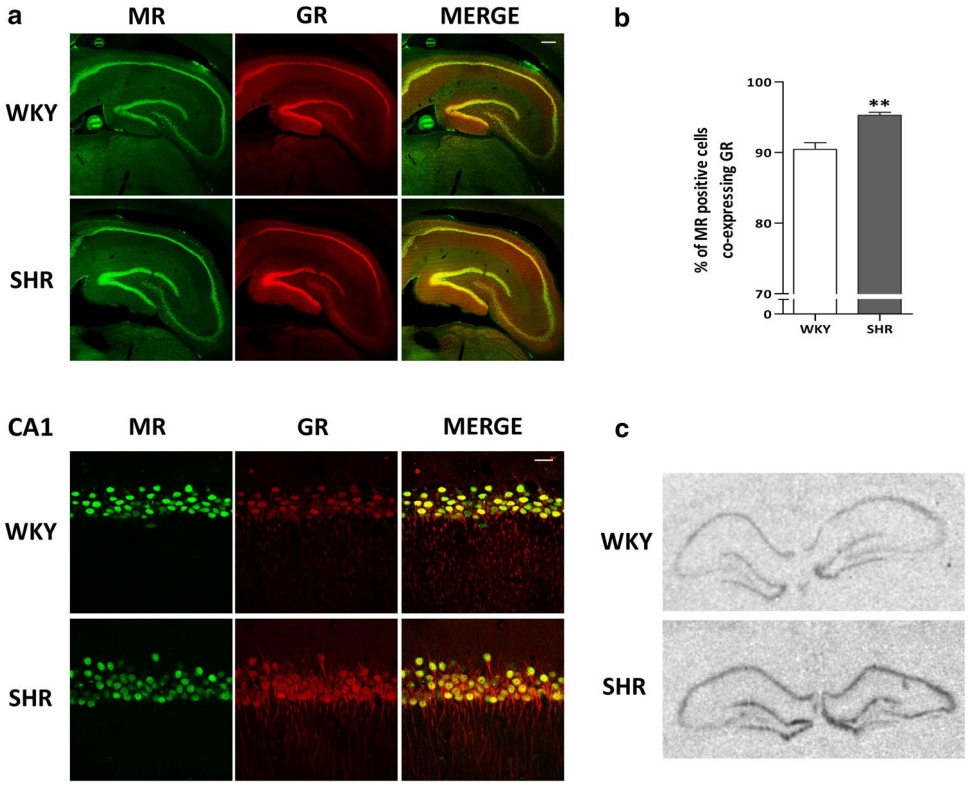

Fig. 1 Expression of the mineralocorticoid receptor (MR) in the hippocampus of spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) normotensive rats. **a**: The upper 6 images represent low power immunofluorescence of MR (green) and the glucocorticoid receptor (GR, red). SHR show increased expression of MR protein vs. WKY rats as well as increased co-localisation of both receptors (merge image, yellow). Inside bar: 600 µm. The lower 6 images in (**a**) correspond to high power view of the CA1 pyramidal cell layer. SHR show increased MR + cells (green) and increased co-localisation of both receptors (merge image, yellow) in this hippocampal region. Inside bar: 60 µm **b**: Quantitative analysis of immunofluorescent MR/GR + cells show higher % of MR + cells co-expressing GR in SHR vs. WKY rats (Student´s "*t*" test : $^{**}p < 0.01$). **c**: Film autoradiograms obtained by in situ hybridisation using a radioactive MR oligonucleotide probe in the dorsal hippocampus. SHR show a stronger signal compared to WKY rats. Reproduced with permision from Brocca et al. (2017) and Pietranera et al. (2012)

hours afterwards, measurement of BP by a tail cuff method show a significantly decreased BP in SHR receiving the anti-MR RU 28318, whereas BP remained unchanged in animals receiving the anti-GR. These data involve MR but not GR in hypertension of SHR and confirms previous reports that blockade of MR with specific antagonists decreases BP (Janiak et al. 1990; Gomez-Sanchez et al. 1992, 2010). However, the MR-containing brain region responsible for the decreased BP following RU 28318 administration remains an open question, because compounds given icv diffuse to many parts of the brain bathed by the cerebrospinal fluid. Accordingly, the circumventricular organs and the area postrema/NTS may be candidates for the hypotensive effect of the MR antagonists.

Expression of Pro-inflammatory Factors in SHR and WKY Rats

Neuroinflammation is a consistent finding of SHR brain, involving several mediators (Sabbatini et al. 2002; Tomassoni et al. 2004; Benicky et al. 2011; Tayebati et al. 2016). Recent findings have shown up-regulation of caspase-1, IL1β, the NLRP-3 inflammasome and NFκB in brain areas controlling BP of SHR, supporting a role of inflammatory factors in hypertension (Avolio et al. 2018). In SHR, overactivation of MR associates with this pro-phlogistic environment involving inflammatory cells such as microglia.

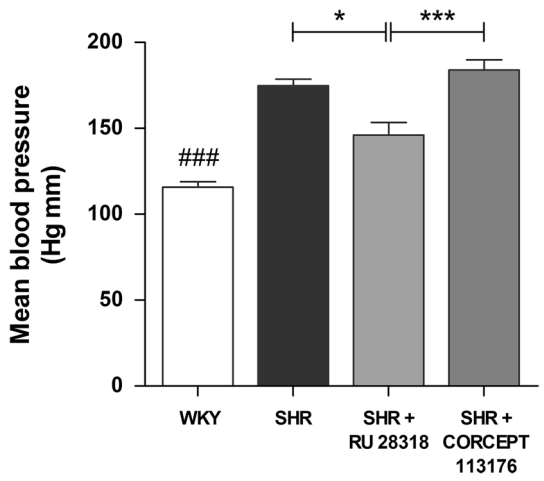

Fig. 2 Effect of central administration of MR and GR antagonists on blood pressure of SHR and WKY rats obtained by tail cuff plethysmography. Anesthetized male rats received icv 400 ng of the MR blocker RU 28318 or the GR blocker CORCEPT 113176 dissolved in 8 µl of 2% ethanol-0.9% NaCl. Controls received vehicle only. Coordinates from Bregma were: lateral: −1.5, anteroposterior: −0.8 and ventral (z) −4.2 mm. Blood pressure was measured 12 h afterwards in vigil animals. Statistical analysis was carried out by ANOVA followed by the Newman-Keuls test: $^{###}p < 0.001$ vs. all groups; $^{*}p < 0.05$ SHR vs. SHR + anti-MR; ***SHR + anti-MR vs. SHR + anti-GR. ($n = 6$ animals per group)

In our study, labelling of microglia with the Iba1 antibody distinguished three main morphological phenotypes: (1) cells displaying a highly ramified processes and small soma, (2) cells showing a hypertrophied soma with less pronounced processes, and (3) a minority group consisting of ameboid-like cells. Counting of total Iba + cells (ramified + hypertrophic + ameboid) have shown higher density in the CA1 and CA3 and hilar region of SHR compared to WKY rats. Quantitatively, higher numbers of ramified and hypertrophic microglia are present in the CA1, CA3 and hilus of the dentate gyrus of SHR vs. WKY (Brocca et al. 2017). In WKY rats, hypertrophic microglia outnumbered the ramified form, whereas in SHR, ramified and hypertrophic microglia distributed equally. Thus, differences in microglia number as well as in the morphological phenotype indicate an activational state of microglia in the hypertensive brain.

As expected, microgliosis of SHR is accompanied by changes in the expression of pro-inflammatory and anti-inflammatory markers. As an indicator of receptor functionality, we have selected the MR and GR-target gene *Sgk1* (Anacker et al. 2013), whereas for neuroinflammation, we have measured the mRNA levels of pro-inflammatory factors including the cyclooxygenase 2 (*Cox2*), *Nlrp3* inflammasome and tumor necrosis factor alpha (*Tnfα*). Conversely, we have also measured *Tgfβ* mRNA levels as a representative anti-inflammatory factor (Qian et al. 2008) and the NADPH-diaphorase activity of nitric oxide synthase (NOS), considering its role in vasodilation and down-regulation after MR activation (Nagata et al. 2006).

Figure 3 shows that the hippocampus of SHR express fivefold higher levels of *Sgk1* mRNA, compared to WKY rats. *Cox2*, an enzyme associated with vascular inflammation in SHR (Renna et al. 2013), increases twofold in the hippocampus of SHR vs. WKY rats, as is the mRNA for the inflammasome component *Nlrp3* (Fig. 3). However, the

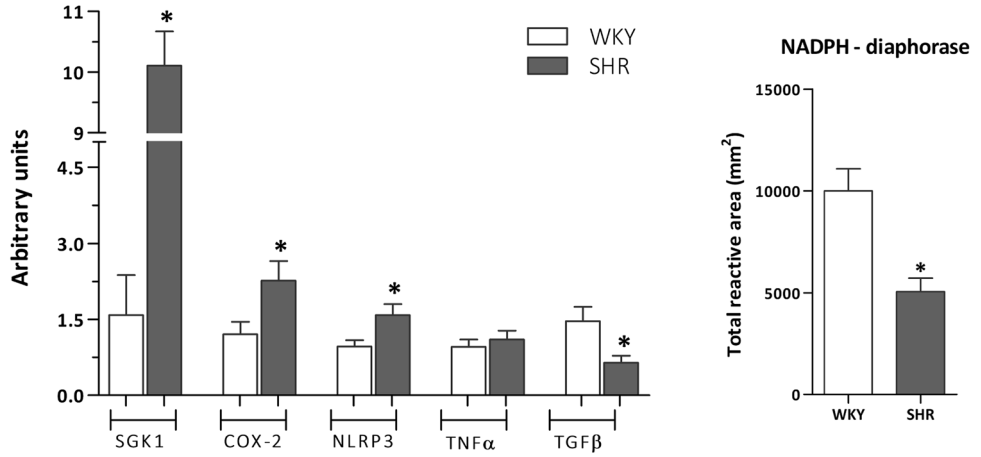

Fig. 3 Real-time PCR analysis of the mRNA of pro-inflammatory, anti-inflammatory factors and vasodilators in the hippocampus of SHR (grey columns) and WKY rats (white columns). mRNA expression, expressed as arbitrary units, showed significant increased levels for serum and glucocorticoid-activated kinase 1 (SGK1), cyclooxygenase 2 (COX2) and NLRP3 subunit of the inflammasome in SHR vs. WKY rats ($^{*}p < 0.05$). No changes were obtained for TNFα, whereas TGFβ and NADPH-diaphorase activity of nitric oxide synthase (rigth hand graph) were decreased in SHR vs. WKY rats ($^{*}p < 0.05$). Statistical analysis by Student's '*t*" test. Reproduced with permission from Brocca et al. (2017)

cytokine *Tnfα* mRNA of SHR is not significantly different compared to WKY rats. In contrast, levels of the anti-inflammatory molecule *Tgfβ* are reduced in SHR vs. WKY rats, and in consonance with previous findings (Hojná et al. 2010), the NADPH-diaphorase activity of NOS becomes significantly lower in the hippocampal CA1 area of SHR vs. WKY rats (Fig. 3). In conclusion, the presence of microgliosis and the imbalance between pro-inflammatory and anti-inflammatory factors and vasodilators reveal an inflammatory status in SHR. This finding is not unique to the hippocampus, as inflammatory mediators are also up-regulated in other brain regions, heart, liver, kidney and vasculature of SHR (DeLano and Schmid-Schönbein 2004; Sun et al. 2006; Tayebati et al. 2016).

Final Remarks

Thus far, our experimental data has demonstrated that increased hippocampal MR expression and enhanced co-localisation with GR associates with a preponderance of pro-inflammatory genes over anti-inflammatory factors. The higher transcription and translation of MR in hippocampus of SHR than WKY rats is in agreement with other studies and can be generalised to hypothalamus, heart, kidney and endothelial vasculature (Mirshahi et al. 1998; DeLano and Schmid-Schönbein 2004). Moreover, the high expression of the inflammatory target *Sgk1* gene, supports the claim that a hyperactive MR behaves as a proverbial "death receptor" in fostering inflammation under adverse conditions (Funder 2004). The enhanced hippocampus MR/GR colocalisation may signal receptor cooperativity at inflammatory genes, possibly through formation of MR:GR heterodimers (Mifsud and Reul 2016).

Oxidative stress caused by tissue damage may play a significant role in the switch from MR physiology into pathophysiology, because it can boost the activation of MR by endogenous glucocorticoids (Mihailidou et al. 2009; Young et al. 2010). Indeed, hippocampal MR binding of corticosterone is increased by about 60% after a systemic or icv challenge with IL-1 together with decreased affinity and increase in circulating corticosterone levels (Schöbitz et al. 1994). This increase in circulating corticosterone and MR capacity, plus the absence of the HSD2 isozyme may increase hippocampal vulnerability and be a conditioning factor for the neuropathology exhibited by SHR. Interestingly, the SHR hippocampus resembles the diabetic skin, a tissue with low expression of the HSD2 isozyme, and where MR activation promotes the release of pro-inflammatory cytokines (Dong and He 2013).

Previous reports have suggested that imbalanced MR:GR stimulation increases hippocampal vulnerability and worsens inflammation (de Kloet et al. 2005; Sorrells et al. 2014; Sasaki and Yoshizaki 2015).The analysis of pro-inflammatory and anti-inflammatory factors in SHR supports this possibility. Of capital importance is the change of microglia phenotype, consistent with a condition of chronic subtle inflammation (Brocca et al. 2017). Thus, we propose a physio-pathological feedforward cascade starting (Fig. 4) with hypertension-induced damage to the vasculature, development of microgliosis and astrogliosis, production of pro-inflammatory mediators and oxidative stress leading to inappropriate MR hyperactivation. Neuronal damage resulting from vasculopathy-induced hypoxia and MR overactivation stimulates release of pro-inflammatory factors from glial cells, which exacerbate oxidative stress and further activates MR.

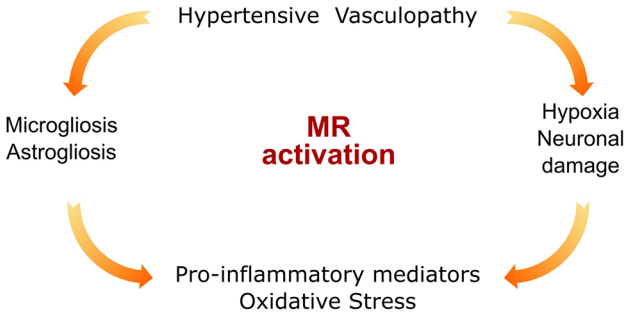

Fig. 4 Diagram suggesting the involvement of MR in hippocampus neuroinflammation. Hypertensive vasculopathy produces microgliosis and astrogliosis, with both cell types sharing responsibility for the increased production of proinflammatory factors and oxidative stress, and hypoxemia with neuronal damage. In both cases, there is inappropriate activation of MR, leading to additional glial activation and production of pro-inflammatory mediators. In this way, the inappropriate activation of the MR becomes part of a vicious cycle causing hippocampal neuropathology in the SHR

However, some questions remain unanswered. As discussed at the beginning of this article, female SHR show lower BP than male SHR. This gender difference may be due to female estrogens, which exert a hypotensive effect or to androgen-induced increase in BP of SHR (Reckelhoff 2001; Pietranera et al. 2008). Additionally, the activity of MR can be modified post-translationally, as shown by the inhibition of histone deacetylases (HDACs). This procedure increases MR acetylation and inhibits the transcription of several MR target genes in SHR (Kang et al. 2015). Further research should disclose if changes in epigenetic mechanisms or receptor expression can explain gender differences in the BP of SHR.

Finally, we recognize that a successful translation requires a correlation with clinical findings. Available reports have shown the intervention of MR in some essential hypertensive patients (Martinez et al. 2009) and in obesity hypertension (Hall et al. 2015) but most are based on the therapeutic value of the MR antagonists eplerenone and spironolactone

(Jeunemaitre et al. 1987; Tam et al. 2017). In view of the emerging participation of MR in human pathology (Jaisser and Farman 2016), it is hoped that preclinical models clarify the association of brain inflammation, oxidative stress and vascular alterations with the activation and/or expression of human MR.

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Author Contributions MEB and LP performed the work and designed the figures; ERK reviewed the manuscript, AFN wrote the paper.

Compliance with Ethical Standards

Conflict of interest The authors report no conflict of interest.

References

Ahima R, Krozowski Z, Harlan R (1991) Type I corticosteroid receptor-like immunoreactivity in the rat CNS: distribution and regulation by corticosteroids. J Comp Neurol 313:522–538

Amenta F, Tayebati SK, Tomassoni D (2010) Spontaneously hypertensive rat neuroanatomy: applications to pharmacological research. Ital J Anat Embryol Arch Ital Anat Ed Embriologia 115:13–17

Anacker C, Cattaneo A, Musaelyan K et al (2013) Role for the kinase SGK1 in stress, depression, and glucocorticoid effects on hippocampal neurogenesis. Proc Natl Acad Sci USA 110:8708–8713

Ando H, Zhou J, Macova M et al (2004) Angiotensin II AT1 receptor blockade reverses pathological hypertrophy and inflammation in brain microvessels of spontaneously hypertensive rats. Stroke 35:1726–1731

Arriza JL, Simerly RB, Swanson LW, Evans RM (1988) The neuronal mineralocorticoid receptor as a mediator of glucocorticoid response. Neuron 1:887–900

Avolio E, Pasqua T, Di Vito A et al (2018) Role of brain neuroinflammatory factors on hypertension in the spontaneously hypertensive rat. Neuroscience 375:158–168

Benicky J, Sánchez-Lemus E, Honda M et al (2011) Angiotensin II AT1 receptor blockade ameliorates brain inflammation. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol 36:857–870

Bohn MC, Howard E, Vielkind U, Krozowski Z (1991) Glial cells express both mineralocorticoid and glucocorticoid receptors. J Steroid Biochem Mol Biol 40:105–111

Brocca ME, Pietranera L, Beauquis J, De Nicola AF (2013) Estradiol increases dendritic length and spine density in CA1 neurons of the hippocampus of spontaneously hypertensive rats: a Golgi impregnation study. Exp Neurol 247:158–164

Brocca ME, Pietranera L, Meyer M et al (2017) Mineralocorticoid receptor associates with pro-inflammatory bias in hippocampus of spontaneously hypertensive rats. J Neuroendocrinol. https://doi.org/10.1111/jne.12

Busillo JM, Cidlowski JA (2013) The five Rs of glucocorticoid action during inflammation: ready, reinforce, repress, resolve, and restore. Trends Endocrinol Metab TEM 24:109–119

Chen J, Gomez-Sanchez CE, Penman A et al (2014) Expression of mineralocorticoid and glucocorticoid receptors in preautonomic neurons of the rat paraventricular nucleus. Am J Physiol Regul Integr Comp Physiol 306:R328–R340

de Kloet ER (2014) From receptor balance to rational glucocorticoid therapy. Endocrinology 155:2754–2769

de Kloet ER, Joëls M (2017) Brain mineralocorticoid receptor function in control of salt balance and stress-adaptation. Physiol Behav 178:13–20

de Kloet ER, Joëls M, Holsboer F (2005) Stress and the brain: from adaptation to disease. Nat Rev Neurosci 6:463–475

DeLano FA, Schmid-Schönbein GW (2004) Enhancement of glucocorticoid and mineralocorticoid receptor density in the microcirculation of the spontaneously hypertensive rat. Microcirculation 11:69–78

Dong F, He X (2013) Pro-nociceptive role of the activation of mineralocorticoid receptor in the pathogenesis of painful diabetic neuropathy. Med Hypotheses 81:436–438

Edwards CR, Stewart PM, Burt D et al (1988) Localisation of 11 beta-hydroxysteroid dehydrogenase–tissue specific protector of the mineralocorticoid receptor. Lancet 2:986–989

Evans LC, Ivy JR, Wyrwoll C et al (2016) Conditional deletion of HSD11b2 in the brain causes salt appetite and hypertension. Circulation 133:1360–1370

Funder JW (2004) Aldosterone, mineralocorticoid receptors and vascular inflammation. Mol Cell Endocrinol 217:263–269

Funder JW (2009) Trilostane, FAD286, and the role of aldosterone in the central regulation of blood pressure: focus on "Role of central nervous system aldosterone synthase and mineralocorticoid receptors in salt-induced hypertension in Dahl salt-sensitive rats". Am J Physiol Regul Integr Comp Physiol 296:R992–R993

Geerling JC, Loewy AD (2006) Aldosterone-sensitive neurons in the nucleus of the solitary: efferent projections. J Comp Neurol 498:223–250

Geerling JC, Engeland WC, Kawata M, Loewy AD (2006) Aldosterone target neurons in the nucleus tractus solitarius drive sodium appetite. J Neurosci 26:411–417

Gómez F, Lahmame A, de Kloet ER, Armario A (1996) Hypothalamic-pituitary-adrenal response to chronic stress in five inbred rat strains: differential responses are mainly located at the adrenocortical level. Neuroendocrinology 63:327–337

Gomez-Sanchez EP (2014) Brain mineralocorticoid receptors in cognition and cardiovascular homeostasis. Steroids 91:20–31

Gomez-Sanchez E, Gomez-Sanchez CE (2014) The multifaceted mineralocorticoid receptor. Compr Physiol 4:965–994

Gomez-Sanchez EP, Fort C, Thwaites D (1992) Central mineralocorticoid receptor antagonism blocks hypertension in Dahl S/JR rats. Am J Physiol 262:E96–E99

Gomez-Sanchez EP, Gomez-Sanchez CM, Plonczynski M, Gomez-Sanchez CE (2010) Aldosterone synthesis in the brain contributes to Dahl salt-sensitive rat hypertension. Exp Physiol 95:120–130

Hall JE, do Carmo JM, da Silva AA et al (2015) Obesity-induced hypertension: interaction of neurohumoral and renal mechanisms. Circ Res 116:991–1006

Hashimoto K, Makino S, Hirasawa R et al (1989) Abnormalities in the hypothalamo-pituitary-adrenal axis in spontaneously hypertensive rats during development of hypertension. Endocrinology 125:1161–1167

Hojná S, Kuneš J, Zicha J (2010) Alterations of NO synthase isoforms in brain and kidney of rats with genetic and salt hypertension. Physiol Res 59:997–1009

Huang BS, White RA, Jeng AY, Leenen FHH (2009) Role of central nervous system aldosterone synthase and mineralocorticoid receptors in salt-induced hypertension in Dahl salt-sensitive rats. Am J Physiol Regul Integr Comp Physiol 296:R994–R1000

Jaisser F, Farman N (2016) Emerging roles of the mineralocorticoid receptor in pathology: toward new paradigms in clinical pharmacology. Pharmacol Rev 68:49–75

Janiak PC, Lewis SJ, Brody MJ (1990) Role of central mineralocorticoid binding sites in development of hypertension. Am J Physiol 259:R1025–R1034

Jeunemaitre X, Chatellier G, Kreft-Jais C et al (1987) Efficacy and tolerance of spironolactone in essential hypertension. Am J Cardiol 60:820–825

Joëls M, de Kloet ER (2017) 30 years of the mineralocorticoid receptor: the brain mineralocorticoid receptor: a saga in three episodes. J Endocrinol 234:T49–T66

Jöhren O, Dendorfer A, Dominiak P, Raasch W (2007) Gene expression of mineralocorticoid and glucocorticoid receptors in the limbic system is related to type-2 like diabetes in leptin-resistant rats. Brain Res 1184:160–167

Kang S-H, Seok YM, Song M et al (2015) Histone deacetylase inhibition attenuates cardiac hypertrophy and fibrosis through acetylation of mineralocorticoid receptor in spontaneously hypertensive rats. Mol Pharmacol 87:782–791

Kenyon CJ, DeConti GA, Cupolo NA, Morris DJ (1981) The role of aldosterone in the development of hypertension in spontaneously hypertensive rats. Endocrinology 109:1841–1845

Koch B, Sakly M, Lutz-Bucher B (1982) Specific mineralocorticoid receptors in the hippocampus of spontaneously hypertensive (SH) rats. I. Evidence for a sex difference. Horm Metab Res 14:166

Konishi A, Tazawa C, Miki Y et al (2003) The possible roles of mineralocorticoid receptor and 11beta-hydroxysteroid dehydrogenase type 2 in cardiac fibrosis in the spontaneously hypertensive rat. J Steroid Biochem Mol Biol 85:439–442

Lopez-Campistrous A, Hao L, Xiang W et al (2008) Mitochondrial dysfunction in the hypertensive rat brain: respiratory complexes exhibit assembly defects in hypertension. Hypertension 51:412–419

Martinez F, Mansego ML, Escudero JC et al (2009) Association of a mineralocorticoid receptor gene polymorphism with hypertension in a Spanish population. Am J Hypertens 22:649–655

Mattson MP, Maudsley S, Martin B (2004) A neural signaling triumvirate that influences ageing and age-related disease: insulin/IGF-1, BDNF and serotonin. Ageing Res Rev 3:445–464

McEwen BS (1994) Corticosteroids and hippocampal plasticity. Ann N Y Acad Sci 746:134–142 178–179

McEwen BS, Weiss JM, Schwartz LS (1968) Selective retention of corticosterone by limbic structures in rat brain. Nature 220:911–912

Mifsud KR, Reul JMHM (2016) Acute stress enhances heterodimerization and binding of corticosteroid receptors at glucocorticoid target genes in the hippocampus. Proc Natl Acad Sci USA 113:11336–11341

Mihailidou AS, Loan Le TY, Mardini M, Funder JW (2009) Glucocorticoids activate cardiac mineralocorticoid receptors during experimental myocardial infarction. Hypertension 54:1306–1312

Mirshahi M, Nicolas C, Agarwal MK (1998) Enhanced activation of the mineralocorticoid receptor in genetically hypertensive rats. Biochem Biophys Res Commun 244:120–125

Müller-Fielitz H, Raasch W (2013) Angiotensin II impairs glucose utilization in obese Zucker rats by increasing HPA activity via an adrenal-dependent mechanism. Horm Metab Res 45:173–180

Nagata D, Takahashi M, Sawai K et al (2006) Molecular mechanism of the inhibitory effect of aldosterone on endothelial NO synthase activity. Hypertension 48:165–171

Oki K, Gomez-Sanchez EP, Gomez-Sanchez CE (2012) Role of mineralocorticoid action in the brain in salt-sensitive hypertension. Clin Exp Pharmacol Physiol 39:90–95

Oster H, Challet E, Ott V et al (2017) The Functional and Clinical Significance of the 24-Hour Rhythm of Circulating Glucocorticoids. Endocr Rev 38:3–45

Pietranera L, Saravia F, Roig P et al (2004) Mineralocorticoid treatment upregulates the hypothalamic vasopressinergic system of spontaneously hypertensive rats. Neuroendocrinology 80:100–110

Pietranera L, Saravia F, Gonzalez Deniselle MC et al (2006) Abnormalities of the hippocampus are similar in deoxycorticosterone acetate-salt hypertensive rats and spontaneously hypertensive rats. J Neuroendocrinol 18:466–474

Pietranera L, Saravia FE, Roig P et al (2008) Protective effects of estradiol in the brain of rats with genetic or mineralocorticoid-induced hypertension. Psychoneuroendocrinology 33:270–281

Pietranera L, Lima A, Roig P, De Nicola AF (2010) Involvement of brain-derived neurotrophic factor and neurogenesis in oestradiol neuroprotection of the hippocampus of hypertensive rats. J Neuroendocrinol 22:1082–1092

Pietranera L, Brocca ME, Cymeryng C et al (2012) Increased expression of the mineralocorticoid receptor in the brain of spontaneously hypertensive rats. J Neuroendocrinol 24:1249–1258

Pineau F, Canet G, Desrumaux C et al (2016) New selective glucocorticoid receptor modulators reverse amyloid-β peptide-induced hippocampus toxicity. Neurobiol Aging 45:109–122

Qian L, Wei S-J, Zhang D et al (2008) Potent anti-inflammatory and neuroprotective effects of TGF-beta1 are mediated through the inhibition of ERK and p47phox-Ser345 phosphorylation and translocation in microglia. J Immunol 181:660–668

Rahmouni K, Barthelmebs M, Grima M et al (2001) Involvement of brain mineralocorticoid receptor in salt-enhanced hypertension in spontaneously hypertensive rats. Hypertension 38:902–906

Ratka A, Sutanto W, Bloemers M, de Kloet ER (1989) On the role of brain mineralocorticoid (type I) and glucocorticoid (type II) receptors in neuroendocrine regulation. Neuroendocrinology 50:117–123

Reckelhoff JF (2001) Gender differences in the regulation of blood pressure. Hypertens Dallas Tex 1979 37:1199–1208

Renna NF, Diez ER, Lembo C, Miatello RM (2013) Role of Cox-2 in vascular inflammation: an experimental model of metabolic syndrome. Mediators Inflamm. https://doi.org/10.1155/2013/513251

Reul JM, de Kloet ER (1985) Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 117:2505–2511

Rotenstein LS, Sheridan M, Garg R, Adler GK (2015) Effect of mineralocorticoid receptor blockade on hippocampal-dependent memory in adults with obesity. Obesity 23:1136–1142

Sabbatini M, Catalani A, Consoli C et al (2002) The hippocampus in spontaneously hypertensive rats: an animal model of vascular dementia? Mech Ageing Dev 123:547–559

Sapolsky RM, Meaney MJ, McEwen BS (1985) The development of the glucocorticoid receptor system in the rat limbic brain. III. Negative-feedback regulation. Brain Res 350:169–173

Sasaki K, Yoshizaki F (2015) Investigation into hippocampal nerve cell damage through the mineralocorticoid receptor in mice. Mol Med Rep 12:7211–7220

Schöbitz B, Sutanto W, Carey MP et al (1994) Endotoxin and interleukin 1 decrease the affinity of hippocampal mineralocorticoid (type I) receptor in parallel to activation of the hypothalamic-pituitary-adrenal axis. Neuroendocrinology 60:124–133

Seckl JR (1997) 11beta-Hydroxysteroid dehydrogenase in the brain: a novel regulator of glucocorticoid action? Front Neuroendocrinol 18:49–99

Shen XZ, Li Y, Li L et al (2015) Microglia participate in neurogenic regulation of hypertension. Hypertension 66:309–316

Sierra A, Gottfried-Blackmore A, Milner TA et al (2008) Steroid hormone receptor expression and function in microglia. Glia 56:659–674

Sorrells SF, Munhoz CD, Manley NC et al (2014) Glucocorticoids increase excitotoxic injury and inflammation in the hippocampus of adult male rats. Neuroendocrinology 100:129–140

Sun L, Gao Y-H, Tian D-K et al (2006) Inflammation of different tissues in spontaneously hypertensive rats. Sheng Li Xue Bao 58:318–323

Sutanto W, Oitzl MS, Rots NY et al (1992) Corticosteroid receptor plasticity in the central nervous system of various rat models. Endocr Regul 26:111–118

Tam TS, Wu MH, Masson SC et al (2017) Eplerenone for hypertension. Cochrane Database Syst Rev. https://doi.org/10.1002/14651858.CD008996.pub2

Tanaka J, Fujita H, Matsuda S et al (1997) Glucocorticoid- and mineralocorticoid receptors in microglial cells: the two receptors mediate differential effects of corticosteroids. Glia 20:23–37

Tayebati SK, Tomassoni D, Amenta F (2016) Neuroinflammatory markers in spontaneously hypertensive rat brain: an immunohistochemical study. CNS Neurol Disord Drug Targets 15:995–1000

Tomassoni D, Avola R, Di Tullio MA et al (2004) Increased expression of glial fibrillary acidic protein in the brain of spontaneously hypertensive rats. Clin Exp Hypertens 26:335–350

Ueno M, Sakamoto H, Tomimoto H et al (2004) Blood-brain barrier is impaired in the hippocampus of young adult spontaneously hypertensive rats. Acta Neuropathol (Berl) 107:532–538

Vallee SM, Grillo CA, Gonzalez S et al (1995) Further studies in deoxycorticosterone acetate treated rats: brain content of mineralocorticoid and glucocorticoid receptors and effect of steroid antagonists on salt intake. Neuroendocrinology 61:117–124

Van Eekelen JA, De Kloet ER (1992) Co-localization of brain corticosteroid receptors in the rat hippocampus. Prog Histochem Cytochem 26:250–258

Van den Berg DT, de Kloet ER, de Jong W (1994) Central effects of mineralocorticoid antagonist RU-28318 on blood pressure of DOCA-salt hypertensive rats. Am J Physiol 267:E927–E933

Wang H-W, Huang BS, Chen A et al (2016) Role of brain aldosterone and mineralocorticoid receptors in aldosterone-salt hypertension in rats. Neuroscience 314:90–105

Yongue BG, Roy EJ (1987) Endogenous aldosterone and corticosterone in brain cell nuclei of adrenal-intact rats: regional distribution and effects of physiological variations in serum steroids. Brain Res 436:49–61

Young MJ, Morgan J, Brolin K et al (2010) Activation of mineralocorticoid receptors by exogenous glucocorticoids and the development of cardiovascular inflammatory responses in adrenalectomized rats. Endocrinology 151:2622–2628