Life Sciences 234 (2019) 116775

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Angiotensin 1 receptor antagonist attenuates acute kidney injury-induced cognitive impairment and synaptic plasticity via modulating hippocampal oxidative stress

Fatemeh Sharifi, Parham Reisi, Maryam Malek*

Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

ARTICLE INFO

Keywords:
AT1R
Ang II
AKI
Synaptic plasticity
Hippocampus
Learning and memory
Losartan

ABSTRACT

Aims: The activation of the angiotensin (Ang) II after acute kidney injury (AKI) triggers oxidative stress and inflammatory cascade which involved not only the kidneys but also the brain. Ang II through the Ang II type 1 receptor (AT1R) may have deleterious effects on hippocampal synaptic transmission and cognitive functions under uremic encephalopathy. The present study was conducted to examine the effects of AT1R antagonist on AKI-induced cognitive and synaptic plasticity impairment.

Main methods: Here, we investigated the effect of AKI and possible pathophysiological roles of AT1R with the selective AT1R antagonist losartan (10 mg/kg/day for consecutive 9 days) on cognitive performance using passive avoidance and Morris water maze tests. In order to understand the synaptic transmission, in vivo short and long-term plasticity were evaluated at the Schaffer collateral-CA1 synapse. Biochemical analysis was also performed to detect possible hippocampal nitric oxide and oxidative stress mechanisms.

Key findings: Our data provide evidence of hippocampal complication following AKI with increased level of nitrite ($P < 0.01$ vs. sham) as well as oxidative stress ($P < 0.01$ vs. sham) that may be responsible for behavioral dysfunction under uremia (spatial memory, $P < 0.001$; passive avoidance $P < 0.01$ vs. sham). Losartan treatment effectively protects against cognitive (spatial memory, $P < 0.01$; passive avoidance $P < 0.05$ vs. AKI-veh) and synaptic plasticity impairments induced by AKI possibly via modulation of oxidative stress in the hippocampus ($P < 0.01$ vs. AKI-veh).

Significance: The present study conclusively demonstrated a protective role of AT1R antagonist losartan in hippocampal complication and neurocognitive dysfunction after AKI via modulating oxidative stress.

1. Introduction

Acute kidney injury (AKI), an abrupt decline in kidney function, is a major cause of morbidity and mortality in hospitalized patients [1,2]. Despite advances in renal replacement therapy, remote organ consequences of AKI are still associated with a high mortality rate in critically ill patients [3,4]. Uremic encephalopathy is an organic brain disorder that develops in patients with acute or chronic renal failure, however the symptoms are more pronounced in acute kidney injury patients than chronic kidney diseases [5,6]. The symptoms ranging from cognitive and memory impairment to delirium, convulsions, and coma [7]. Brain-kidney crosstalk may explain cerebral involvement under uremic conditions through different identified pathways including inflammatory cascades [8], induction of brain oxidative stress [9] and elevated nitric oxide (NO) [10,11]. Hippocampal cellular inflammation and functional changes have been observed following AKI [12]. However, the effects of acute renal damage on memory and cognitive functions of the hippocampus have not yet been studied. Activation of renin-angiotensin (Ang) II system is a well-established pathway and plays a pivotal role in progressive deterioration of ischemic AKI [13]. Uremic toxins may be directly responsible for the activation of intrarenal renin-Ang II system [14,15]. Ang II type1 receptor (AT1R) is expressed in diverse brain regions, including the hippocampus and as the predominant Ang II receptors in the kidneys [16,17]. While under normal conditions the blood–brain barrier (BBB) restricts peripheral Ang II to entering the brain regions, inflammatory states such as that seen in AKI may contribute to the BBB integrity disruption [18,19]. Some evidences suggest the efficacy of AT1R antagonists to halt the progression of AKI [20,21]. Furthermore, AT1R activation is responsible for the brain oxidative stress and cognitive

* Corresponding author.
E-mail addresses: malek_m57@yahoo.com, malek.maryam@med.mui.ac.ir (M. Malek).

https://doi.org/10.1016/j.lfs.2019.116775
Received 21 June 2019; Received in revised form 12 August 2019; Accepted 15 August 2019
Available online 16 August 2019

impairment [16]. Angiotensin II, through AT1R, is a prominent activator of the NADPH-oxidase complex, and NADPH oxidase-mediated generation of reactive oxygen species contribute the main cause of oxidative stress and inflammatory processes that are involved in cognitive deficits in some neurodegenerative diseases [16,22]. Brain oxidative stress and increased nitric oxide production have been implicated as the major mediators of neurological complications in uremic states [11,23] which was associated with spatial working memory dysfunction in a mouse model of chronic uremia [24]. Blockade of AT1 receptors has been shown protective effects on brain damage and cognitive impairment in ischemic brain [25], salt-induced hypertensive rats [26] and stroke-prone spontaneously hypertensive rats [27]. Losartan has also been shown to ameliorate alterations of the neuronal activity in some brain areas induced by chronic kidney injury [28].

Thus, it seems that the AT1R activation following AKI may mediate the kidney-brain crosstalk in uremic encephalopathy. In the present study, we examined the electrophysiological, behavioral and biochemical aspects of uremic conditions of AKI and possible involvement of AT1 receptors in the pathophysiology of the uremic encephalopathy.

2. Material and methods

2.1. Subjects and experimental groups

Male Wistar rats (n = 40, BW: 200–250 g) were housed in groups of 5 in a polyethylene cages and kept under controlled temperature conditions (22 ± 3 °C) with a 12 h-light/dark cycle and free access to water and food. The experimental procedures were approved by the Ethics Committee of the Isfahan University of Medical Sciences in accordance with ethical guidelines for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA).

The animals were randomly distributed into four experimental groups of ten rats each; sham-operated rats (sham), rats with AKI model (AKI), animals received AT1R antagonist losartan after AKI induction (AKI + Los) and animals received 0.9% sodium chloride solution (AKI + Veh) after AKI induction.

2.2. Experimental procedures

A schematic illustration showing time lines for the whole experimental design was depicted in Fig. 1.

The treatment groups received intraperitoneal (i.p.) injection of AT1R antagonist losartan (10 mg/kg/day, Sigma-Aldrich) or vehicle (saline) for 9 consecutive days [29,30] from one day before AKI induction.

2.2.1. Induction of AKI

Bilateral renal ischemia reperfusion is a well-established model of AKI and its efficacy was also approved in our previous studies [31]. AKI was induced by bilateral clamping of the renal pedicles using non-traumatic vessel clips for 45 min via two small flank incisions under xylosine (5 mg/kg) and ketamine (100 mg/kg) anesthesia (i.p.). Occlusion of blood supply was confirmed by color change in the kidney. Ischemic phase was followed by clamp removal and visually confirmed reperfusion. The skin and muscle were sutured, and the rats were allowed to recover for one week. The rats in the sham-operated group underwent the same surgical procedure except for clamping of the renal pedicles. Renal function was assessed by blood sampling from the tail vein at 24 h after the onset of reperfusion (based upon renal function tests (BUN and creatinine), when the peak injury is observed approximately 24 h following ischemia) [32,33].

2.3. Behavior assessment

2.3.1. Morris water maze (MWM) test

Spatial memory was investigated in the MWM which consists of 4 consecutive acquisition sessions and memory retention or one probe trial (Day 5) that took place 24 h after the last acquisition session. The apparatus consists in a circular black-painted metal pool (180 cm in diameter) filled with water (21 ± 2 °C) and surrounded by various spatial cues. The pool was arbitrarily divided into four quadrants (North, South, West, and East). Rats were first trained to find the invisible submerged platform at fixed position in the southeast quadrant for four 60-s trials per day with an intertrial interval of 30 s during acquisition session. In each trial, the rat was released randomly from one of the four pool quadrant and remained on the platform between each trial for 30 s. Latency to reach the escape platform was tracked for each trial by a video camera mounted above the pool. To assess memory retention, the platform was removed and the rats were allowed to swim for one 60 s probe trial on the fifth day. Time spent in target quadrant was evaluated and compared between groups [34–36].

2.3.2. Passive avoidance test

The passive avoidance task was measured to evaluate memory performance in a fear-motivated test using the shuttle box, in which the rats were conditioned in a single trial inhibitory avoidance task on day 1 and tested for retention of the task 24 h later. The apparatus consisted of two equal sizes compartments (50 × 30 × 25 cm), the black poorly illuminated compartment and a white illuminated compartment, were separated by a guillotine gate. In the training phase, the rats were placed in the white compartment for 30 s, then the guillotine gate was opened and initial latency to enter the black compartment was recorded. When the rats innately entered the dark compartment, the guillotine gate was closed and an inevitable mild electric foot shock was delivered (1 mA, 2 s). During the testing phase on day 2, the rats were again placed into the white compartment and the latency to step through to the dark compartment was recorded (maximum 300 s) [37].

2.4. Electrophysiological recordings

2.4.1. Field excitatory postsynaptic potential (fEPSP)

The animal's head was fixed to the stereotaxic frame and the skull surface was exposed under deep urethane anesthesia (1.6 g kg^{-1}, i.p.).

Extracellular field potentials were recorded by inserting a bipolar stainless steel microelectrode (0.125 mm in diameter; Advent, UK) in the Schaffer collateral pathway of the right hippocampus (AP, 3; ML, 3.5; DV, 2.8–3), and a unipolar recording microelectrode into the ipsilateral CA1 area (AP, 4.1; ML, 3; DV, 2.5) [38]. Synaptic field potentials in the CA1 area were evoked by stimulating the Schaffer collateral pathway. The test stimulation intensity was chosen to elicit an EPSP that is approximately 50% of the maximum response recorded during the input/output (I–O) function.

2.4.2. Paired-pulse response (PPR)

Basal neurotransmission and release probability were determined from the paired-pulse responses by applying repetitive twin equal intensity pulse stimulation at interpulse interval (IPI) of 50 ms. The paired-pulse index (PPI) was calculated as the ratio of the average of three subsequent responses of interstimulus interval to the average of

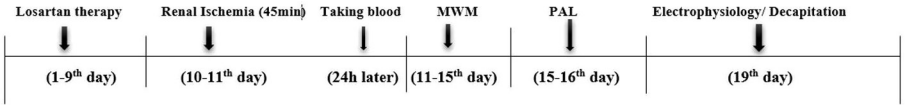

Fig. 1. Experimental design of the study.

three previous responses.

2.4.3. Long-term potentiation (LTP)

LTP was delivered after a 30 min baseline recording period by applying one train of high-frequency stimulation (100 Hz, four trains of 50 pulses, 0.15 ms stimulus duration, and 10 s interburst interval) and was calculated relative as percentage change of the amplitude or slope from the average pre-HFS response that was recorded over the 90 min after tetanic stimulation.

2.5. Histological examination

At the end of the experiment, the animals were killed by decapitation under urethane anesthesia, the right hemisphere was excited and fixed in 10% buffered formalin solution for at least 10 days. To verify electrode placement histologically, five μm thick transverse sections were cut using a freezing microtome (Fig. 8). Then the whole hippocampus was removed from the left hemisphere and stored at −80 °C for the biochemical assessment.

2.6. Biochemical assessment

2.6.1. Renal function assessment

Blood samples (0.5 ml) were obtained from the tail vein 24 h after reperfusion to determine the levels of urea and creatinine (Cr) and the sera were separated by centrifugation at 10,000 ×*g* for 3 min. The levels of serum BUN and Cr were evaluated using quantitative diagnostic kits (Pars Azmoon, Iran).

2.6.2. Lipid peroxidation measurements in the hippocampus

In order to determine the degree of lipid peroxidation, malondialdehyde (MDA) was measured based on the reaction with thiobarbituric acid (TBA) to produce a pink-colored pigment with a 532 nm absorption maximum [9]. A mixture of 1 ml of 10% trichloroacetic acid in 1 ml of 0.67% thiobarbituric acid was added to hippocampal homogenate (10% w/v) and kept in boiling water bath for 45 min. After cooling, the mixture was centrifuged at 1000 ×*g* for 10 min, and its absorbance was measured against reagent blank containing all components except the sample. The concentration of MDA was expressed and calculated as follows: nmol of MDA per gram of tissue = *Absorbance* / (1.65 × 105).

2.6.3. Nitrite content determination in the hippocampus

The nitrite production was determined based on the Griess reaction [39]. Briefly, 100 μl of hippocampal homogenate supernatant (10% w/v) was incubated with the same volume of Griess reagent containing 1% sulfanilamide, 0.1% naphthalene diamine dihydrochloride, and 2.5% phosphoric acid at room temperature for 10 min. The absorbance was determined at 520 nm and nitrite content was calculated from a standard nitrite curve created by using sodium nitrite standards.

2.7. Statistical analysis

All analyses were performed using the SPSS version 21 for windows (IBM Corporation). Training trials data in MWM and data from LTP induction and maintenance were analyzed by repeated measures ANOVA followed by Tukey; and the data from paired pulse facilitation, probe day of MWM and biochemical were analyzed by one-way ANOVA followed by Tukey. All data were expressed as mean ± SD. The value of $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Body weight

Fig. 2 shows the average body weights of the rats at the beginning and end of the study. There was no significant difference in body weight between the groups.

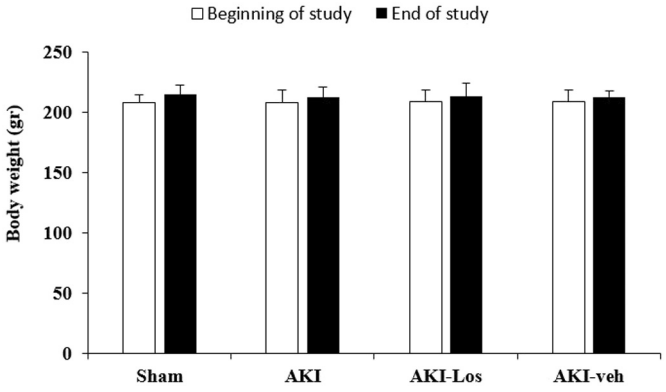

Fig. 2. Comparison of body weight change between rats at the beginning and end of the study. Values represent mean ± SD for ten rats in each group.

3.2. Spatial learning and memory assessment

In order to assess hippocampus-dependent learning and memory, we measured the latency to find the platform over four training days (blocks), and time spent in the goal quadrant during a probe trial on the fifth day in the MWM task. A repeated-measures ANOVA indicated a significant decrease in escape latency to acquire hidden platform in all experimental groups during acquisition trials (BLOCK effect, $F(3, 102) = 206.12$, $P < 0.001$; Fig. 3A), this pattern of reduction in latencies to acquire hidden platform across the blocks was not significantly different between the groups (Block effect, $F(9, 102) = 0.37$, $P = 0.94$; Fig. 3A), means that all rats learned equally well the task during the training days.

In the probe-trial test, one-way ANOVA revealed that the AKI (23.88 ± 12.4 s and 45.96 ± 7.5 s respectively; $P < 0.001$) and AKI-veh (19.97 ± 12.2 s and 45.96 ± 7.5 s respectively; $P < 0.001$) rats spent a short time in the target quadrant compared to sham rats. However, losartan treatment improved memory deficits versus vehicle-treated rats (30.66 ± 9.2 s and 19.97 ± 12.2 s respectively; $P < 0.01$) (Fig. 3B).

3.3. Passive avoidance memory assessment

As illustrated in Fig. 4, the mean initial latencies to enter the dark compartment during training were the same in all groups, whereas AKI (91.67 ± 118.8 s and 246 ± 106.3 s respectively; $P < 0.01$) and AKI-veh rats (114.78 ± 130.2 s and 246 ± 106.3 s respectively; $P < 0.05$) showed a significant deficit in retention latencies compared to the sham group. However, losartan treatment attenuated AKI-induced memory deficits versus AKI (217.8 ± 92.48 s and 91.67 ± 118.8 s respectively; $P < 0.05$) and AKI-veh rats (217.8 ± 92.48 s and 246 ± 106.3 s respectively; $P < 0.05$).

3.4. Electrophysiology

3.4.1. Long-term potentiation

LTP was induced by high-frequency stimulation (100 Hz) at a stimulus intensity that evoked a fEPSP of approximately 80% of the maximum response. A repeated measures ANOVA revealed that fEPSP amplitude (Time effect, $F(8, 256) = 32.29$, $P < 0.001$; Fig. 5A) and fEPSP slope (Time effect, $F(8, 264) = 30.45$, $P < 0.001$; Fig. 5B) decreased in all groups. AKI induced by bilateral renal ischemia reperfusion in AKI-veh and AKI rats showed a significant decline in fEPSP amplitude (Fig. 5A) and slope (Fig. 5B) versus sham rats. Nevertheless, losartan significantly increased fEPSP amplitude (Fig. 5A) and slope

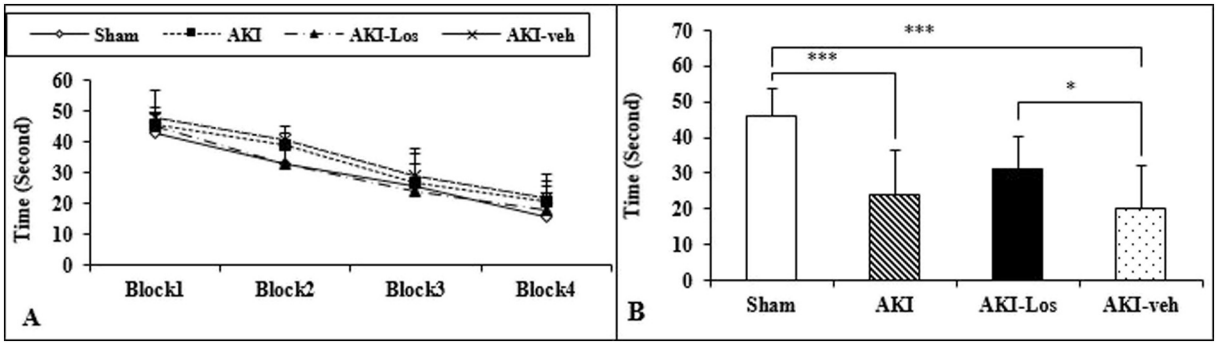

Fig. 3. (A) Escape latency to reach the platform in each block (day) during 4 training days or acquisition trials. Each block is the average of four consecutive trials. (B) Time spent in target quadrant during the probe trial on day 5. Data are expressed as mean ± SD (n = 10). *P < 0.05, ***P < 0.001.

(Fig. 5B) compared to that in the vehicle treated group during 70 min after the tetanic stimulation.

3.4.2. Paired pulse responses

In order to identify the presynaptic involvement in the expression of long-term plasticity, we evaluated the paired-pulse responses at the Shaffer collateral to CA1 synapses. Paired-pulse facilitation, a form of short-term plasticity, is associated with increased probability of presynaptic release and indicates the presynaptic involvement in long-term potentiation. Fig. 6A, B respectively shows evoked fEPSP amplitude and slope in CA1 neurons by paired-pulse stimulations at inter-pulse interval of 50 ms. one way ANOVA showed significant depression in fEPSP amplitude (73.3 ± 22 s and 40.8 ± 23.8 s sham versus AKI; P < 0.05), (73.3 ± 22 s and 46.4 ± 17.72 s sham versus AKI-veh; P < 0.05) and fEPSP slope (92.6 ± 28.4 s and 53.2 ± 40.45 s sham versus AKI; P < 0.05) in AKI and AKI-veh rats compared to control groups. However, administration of losartan showed a significant facilitation in fEPSP slope (99.5 ± 31.65 s and 53.2 ± 40.45 s AKI-Los versus AKI; P < 0.05), (99.5 ± 31.65 s and 54.6 ± 10.3 s AKI-Los versus AKI-veh; P < 0.05) compared to AKI and AKI-veh rats.

3.5. Biochemical

The serum nitrogen or blood urea nitrogen (BUN) and creatinine levels, which are indicators of kidney function, are shown in Fig. 7. Bilateral renal ischemia reperfusion injury induced an increase in serum BUN (23.89 ± 4.42 s and 45.88 ± 19.9 s sham versus AKI, P < 0.01), (23.89 ± 4.42 s and 45.70 ± 14.6 s sham versus AKI-veh, P < 0.01), and creatinine (0.48 ± 0.1 s and 0.7 ± 0.24 s sham versus AKI, P < 0.01), (0.48 ± 0.1 s and 0.82 ± 0.1 s sham versus AKI-veh, P < 0.01) levels at 24 h after reperfusion. Meanwhile, treatment with the losartan ameliorated serum levels of BUN (32.52 ± 7 s and 45.70 ± 14.6 s AKI-Los versus AKI-veh, P < 0.05), (32.52 ± 7 s and 45.88 ± 19.9 s AKI-Los versus AKI, P < 0.05) and creatinine (0.53 ± 0.09 s and 0.82 ± 0.1 s AKI-Los versus AKI-veh, P < 0.001), (0.53 ± 0.09 s and 0.7 ± 0.24 s AKI-Los versus AKI, P < 0.01) compared to the vehicle treated group.

Subsequently, we investigated the influence of AKI and losartan treatment on the production of MDA as a marker of lipid peroxidation in the hippocampus tissue.

As displayed Fig. 7, there was a marked increase in MDA in the AKI (16.09 ± 14.04 s and 2.69 ± 1.93 s AKI versus sham, P < 0.01), and AKI-veh (19.1 ± 9.8 s and 2.69 ± 1.93 s AKI-veh versus sham, P < 0.01) groups compared with sham group. The MDA levels markedly decreased in rats treated with losartan intervention compared with AKI-veh (7.1 ± 7.83 s and 19.1 ± 9.8 s AKI-Los versus AKI-veh, P < 0.01) and AKI (7.1 ± 7.83 s and 16.09 ± 14.04 s AKI-Los versus AKI, P < 0.05) rats.

Additionally, hippocampal nitrite (the oxidation product of NO) levels were measured to determine whether NO levels in the hippocampus were affected by AKI and losartan. The results showed that the nitrite content in the hippocampal tissue was significantly increased in the AKI group compared with those in the control group (1.1 ± 0.35 s and 0.7 ± 0.27 s AKI versus sham, P < 0.01; Fig. 7). However, treatment with losartan had no significant effect on nitrite levels (Fig. 7).

4. Discussion

The present study was designed to evaluate the synaptic plasticity of hippocampal neurons and memory performance 9 days after losartan treatment in animal model of AKI. The findings generally revealed that losartan as a selective inhibitor of AT1R dramatically increases paired-pulse facilitation, a form of short-term synaptic plasticity, and long-term potentiation of hippocampal dentate gyrus neurons. Our behavioral studies also showed improvement in learning and memory using Morris water maze for spatial long-term memory and step-through type passive avoidance task to examine short-term memory based on the

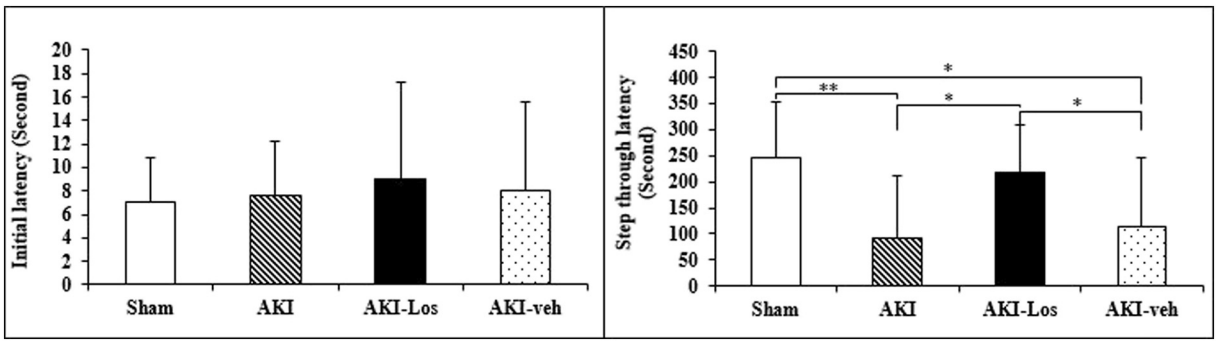

Fig. 4. Initial latency before (left) induction of electrical shock and 24 h after the induction of electrical shock (right) in four groups of passive avoidance test. The values presented are the means ± SD of three independent experiments (n = 10). *P < 0.05.

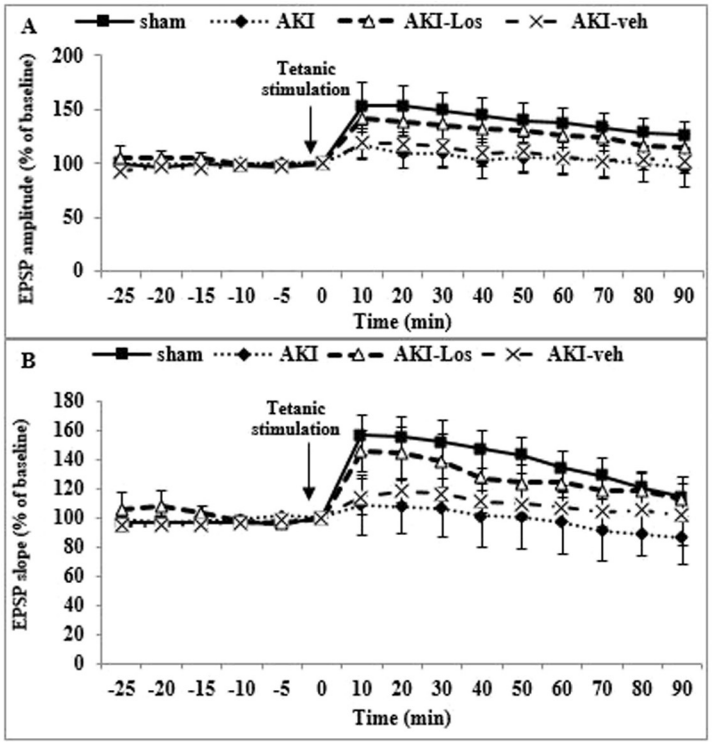

Fig. 5. Time-course of percentage change in the (A) amplitude and (B) slope of long-term synaptic plasticity of CA1 neurons post-100 Hz tetanic stimulation of Schaffer collaterals. (C) Sample traces show fEPSPs recorded in hippocampal CA1 neurons before and after LTP induction. Data are presented as percentage change from baseline EPSP and expressed as mean ± SD (n = 10).
A) $P < 0.05$ AKI-Los versus AKI-veh and AKI-Los versus AKI at 10–70 min, $P < 0.01$ sham versus AKI at 10 and 50–90 min, $P < 0.001$ sham versus AKI at 20–40 min, $P < 0.01$ sham versus AKI-veh at 10–70 min.
B) $P < 0.05$ AKI-Los versus AKI-veh at 10–30 min, $P < 0.05$ AKI-Los versus AKI at 30-40 min, $P < 0.01$ AKI-Los versus AKI at 10–20 min, $P < 0.01$sham versus AKI at 10–70 min, $P < 0.05$sham versus AKI at 80–90 min, $P < 0.01$ sham versus AKI-veh at 10–60 min, $P < 0.05$ sham versus AKI-veh at 70 min.

negative reinforcement.

Activation of intrarenal renin angiotensin and upregulation of angiotensinogen and Ang II type 1a receptor in injured kidneys was described previously [40]. In addition to systemic vascular and renal effects of angiotensin II, peripheral Ang II can enter the brain via the blood-brain barrier deficient circumventricular organs or BBB disruption situations to increases cerebellar oxidative stress with deleterious effects on cognitive functions by acting on AT1R [22]. Moreover, uremia may alter the expression of Ang II in the brain. Recently, Haruyama et al. have reported increased expression of Ang II in the brain of mouse model of chronic kidney disease, which was suppressed by systemic administration of an Ang II receptor antagonist [41]. Neuronal pathway independent of systemic Ang II in ischemic AKI may also be involved in this reno-cerebral connection [42]. Hyperactivation of AT1R by high levels of brain Ang II precedes the release of proinflammatory or inflammatory cytokines in the brain [43]. Additionally, accumulation of uremic toxins in renal failures significantly associated with elevated AT1R expression and alteration in ionic currents with the impact on the action potential duration [14,44]. Nitric oxide is another significant mechanism which plays a critical role during renal disease and oxidative stress. High amounts of NO production through inducible or immunological NO synthase in response to pro-inflammatory

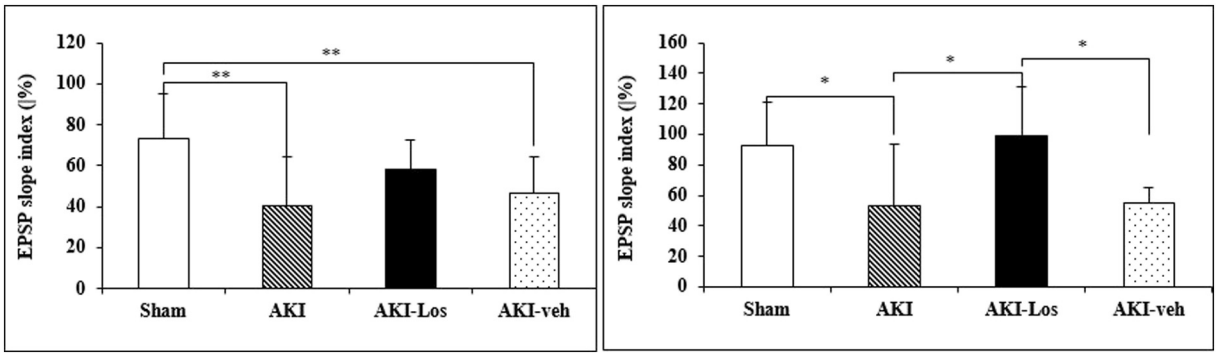

Fig. 6. Paired-pulse facilitation induction of fEPSPs recorded in the CA1 area after stimulation of Schaffer collaterals (A) The fEPSP amplitude ratio (percentage of mean Amp2 / Amp1), and (B) fEPSP slope ratio (percentage of mean fEPSP2 / fEPSP1). PPR was tested at 50, ms IPI between groups. Data are expressed as mean ± SD (n = 10). *$P < 0.05$, **$P < 0.01$.

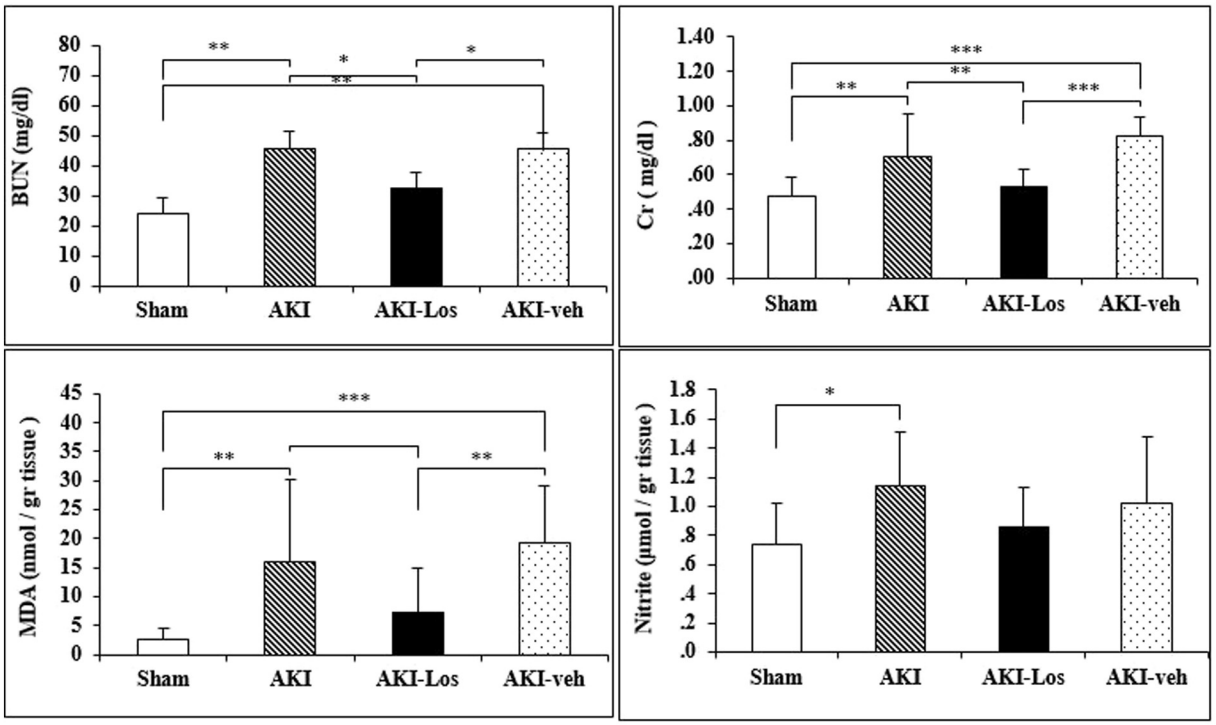

Fig. 7. Serum BUN and creatinine levels at 24 h following reperfusion *P < 0.05, **P < 0.01, and ***P < 0.001. MDA and nitrite concentration in the hippocampus of different studied groups. Values are given as mean ± SD from each group (n = 10). *P < 0.05 and **P < 0.01.

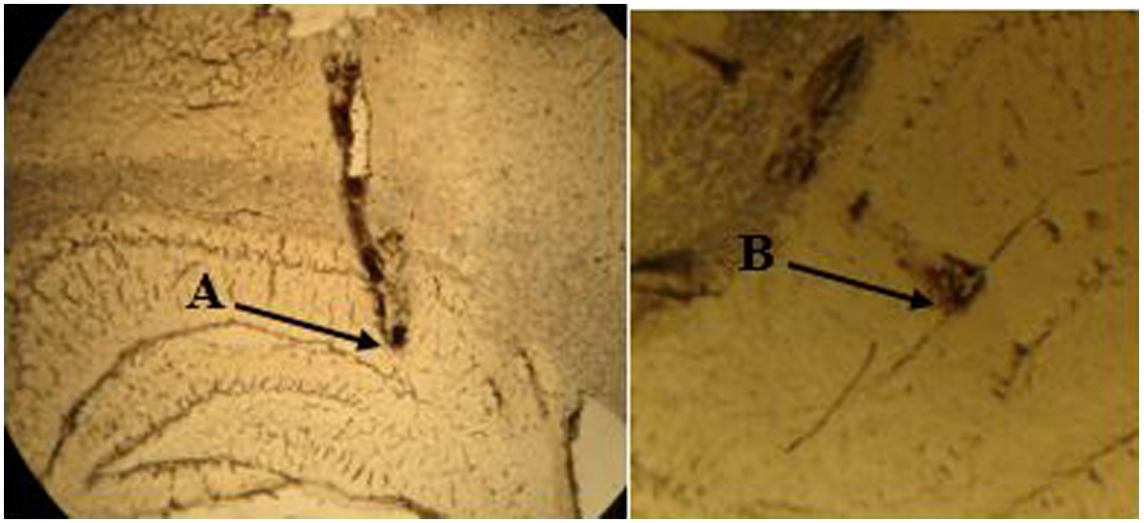

Fig. 8. Coronal section of hippocampus showing tips of the stimulating and recording electrodes in the (A) Schaffer collateral pathway and (B) hippocampal area CA1.

cytokines or endotoxins during inflammatory states such as that seen in AKI cause systemic vasodilatation and leads to increased sympathetic activity and more angiotensin production [45]. It also seems likely that NO derived from inducible NO synthase may have neurotoxic effects [46]. These observations are generally consistent with our reports indicated that AKI leads to oxidative stress and increased nitrite level, an end product of NO, in the hippocampus. As expected, hippocampal involvement in AKI is associated with negatively affected of long-term memory and cognitive functions; however, these effects can be reversed by losartan via augmenting hippocampal antioxidant defense capacity. Our finding confirmed the deleterious effect of AKI on the hippocampus as a remote organ. Manchang Liu and et al. investigated the brain inflammation and locomotor activity in mice undergoing renal ischemia and found enhanced vascular and BBB permeability with increased microglial cells and pyknotic neuronal cells in the hippocampus [12]. Thus, AKI could target the hippocampus as a prime area of cellular brain inflammation due to the production of soluble inflammatory proteins and disruption of BBB. Increased BBB permeability following AKI allows penetrating cytokines into the brain with consequences of edema and inflammation. Furthermore, cytokines activate the microglia which amplify the BBB disruption and further impact on brain function [47]. On the other hand, a higher permeability of the BBB allowing the entry of circulating Ang II within the brain where especially rich in AT1 and AT2 receptors. Ang II was shown to inhibit LTP in perforant path-stimulated dentate granule cells which could be blocked with losartan [48,49]. Losartan treatment has also shown therapeutic

effects not only on blood pressure control but also on cognitive function in elderly hypertensive patients [50]. In this regard, an association was observed between cognitive performance and antihypertensive drugs that act via the renin–angiotensin system [51–53]. An elevation of AT1Rs and AT1R activity are observed in neurons of spontaneous hypertensive rat (SHR) [54]. Recent studies in Dahl salt-sensitive hypertensive and spontaneously hypertensive rats with overactive brain AT1 receptors and cognitive deficits have reported that AT1R blockade ameliorated BBB disruption in the hippocampus, cognitive deficits and brain inflammation [55–57]. Our analysis also revealed that hippocampal involvement in AKI as a critical area implicated in learning and memory can lead to cognitive and synaptic plasticity impairment as seen in AKI group and losartan promotes brain plasticity and prevents memory loss.

Renoprotective effects of AT1R blockers are well known in acute or chronic kidney diseases and have been shown in some human and experimental nephropathies [20,21,58–60]. These results are consistent with the results we obtained in this study, where clearly indicate that losartan has beneficial effects on the biochemical parameters of kidney function by normalization of serum levels of creatinine and BUN. The possible main mechanisms underlying its nephroprotective effects are anti-inflammatory, and antioxidant properties by blocking the proliferation and activation of leukocytes and chemokines that may be directly stimulated by Ang II [61–63]. It seems that brain inflammation due to peripheral or central inflammatory conditions to be strongly associated with AT1R function [64]. Current evidence suggests that neuronal function may be positively modulated by AT1R antagonists [65]. The AT_1 receptors are densely expressed across many organs including the kidney and vasculature, and throughout many brain regions [17,66]. Losartan was shown to improve cognitive performance using inhibitory shock avoidance paradigm which may be due to decreased in endogenous Ang II activity in the brain [67]. Losartan may also prevent BBB dysfunction possibility via a direct effect on endothelial cells of brain vessels [68,69]. An analysis of a large, prospective US cohort, involving mostly elderly male cardiovascular patients, revealed that angiotensin receptor blockers administration reduces cognitive impairment and progression of Alzheimer's disease [70]. Acute administration of losartan has also been shown cognitive-enhancing potential in normotensive young adults on a task of prospective memory and highly effective in reversing the detrimental effects of scopolamine in a standard lexical decision paradigm [71]. Here we further showed that losartan can influence synaptic transmission and short-term plasticity at the Shaffer collateral to CA1 synapses by enhancing of paired-pulse facilitation of excitatory postsynaptic potentials. Paired pulse facilitation is highly dependent on and inversely correlated to the basal release probability of a synapse. Low initial release probability tend to show short-term facilitation, whereas high release probability synapses display short-term depression. An increase in paired-pulse ratio of the fEPSP slope as seen in losartan treated group indicates a lower release probability and may reflect synaptic efficacy through the release probability of the presynaptic cell by effective number of transmitter release sites and persistence of residual calcium in the presynaptic terminal. No significant change in paired pulse amplitude occasionally indicate no change in the number of released vesicles. Taken together, our findings demonstrate that AKI induces negative effects on both behavior and synaptic plasticity which can be reversed by losartan treatment via modulating hippocampal oxidative stress. Therefore, AT1R blockers may be useful as a pharmacological target in the treatment of AKI-induced cognitive impairment among critically ill patients.

There are several limitations in the present study. First, we did not determine angiotensin II levels and AT1R expression as well as changes in CA1 neurons in the hippocampus. Second, we measured oxidative stress only in the hippocampus, and did not determine whether or not losartan reduced oxidative stress in other sites of the brain. Third, we could not determine the dose dependency of losartan and changes in permeability of the blood-brain barrier under AKI condition for penetrating losartan into the hippocampus. Finally, we used losartan given systemically to blockade AT1R in the hippocampus. The possibility that losartan may have affected other brain areas and systemic organs cannot be excluded.

5. Conclusions

AT1R activation in AKI may mediate hippocampal oxidative stress and subsequent cognitive deficits following AKI. Blockade the AT1R by losartan appears to be an effective strategy to improve cognition and synaptic plasticity in acute renal failure. Perhaps future clinical trials targeting the AT1R are necessary to verify the usefulness of these agents in treating cognitive impairment under uremic encephalopathy.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors wish to express their thanks to Professor Mehdi Nematbakhsh for the generous supply of losartan.

Funding

This work was supported by the Isfahan University of Medical Sciences (Grant number 196133 to M.M.).

References

[1] P.K. Moore, R.K. Hsu, K.D. Liu, Management of acute kidney injury: core curriculum 2018, Am. J. Kidney Dis. 72 (1) (2018) 136–148.
[2] T. Ali, et al., Incidence and outcomes in acute kidney injury: a comprehensive population-based study, J. Am. Soc. Nephrol. 18 (4) (2007) 1292–1298.
[3] G.P. Kaushal, S.V. Shah, Challenges and advances in the treatment of AKI, J. Am. Soc. Nephrol. 25 (5) (2014) 877–883 (ASN. 2013070780).
[4] C.-C. Shiao, et al., Long-term remote organ consequences following acute kidney injury, Crit. Care 19 (1) (2015) 438.
[5] D. Burn, D. Bates, Neurology and the kidney, J. Neurol. Neurosurg. Psychiatry 65 (6) (1998) 810–821.
[6] P. De Deyn, et al., Clinical and pathophysiological aspects of neurological complications in renal failure, Acta Neurol. Belg. 92 (4) (1992) 191-120.
[7] G. Bucurescu, Neurological Manifestations of Uremic Encephalopathy Clinical Presentation, Med Scape (2016).
[8] A.S. Miranda, et al., Kidney–brain axis inflammatory cross-talk: from bench to bedside, Clin. Sci. 131 (11) (2017) 1093–1105.
[9] A. Kovalčíková, et al., Oxidative stress in the brain caused by acute kidney injury, Metab. Brain Dis. 33 (3) (2018) 961–967.
[10] A. Davenport, The brain and the kidney–organ cross talk and interactions, Blood Purif. 26 (6) (2008) 526–536.
[11] P. De Deyn, R. Vanholder, R. D'Hooge, Nitric oxide in uremia: effects of several potentially toxic guanidino compounds, Kidney Int. 63 (2003) S25–S28.
[12] M. Liu, et al., Acute kidney injury leads to inflammation and functional changes in the brain, J. Am. Soc. Nephrol. 19 (7) (2008) 1360–1370.
[13] I.M. van der Meer, P. Cravedi, G. Remuzzi, The role of renin angiotensin system inhibition in kidney repair, Fibrogenesis Tissue Repair 3 (1) (2010) 7.
[14] C.-Y. Sun, S.-C. Chang, M.-S. Wu, Uremic toxins induce kidney fibrosis by activating intrarenal renin–angiotensin–aldosterone system associated epithelial-to-mesenchymal transition, PLoS One 7 (3) (2012) e34026.
[15] H. Shimizu, et al., CREB, NF-κB, and NADPH oxidase coordinately upregulate indoxyl sulfate-induced angiotensinogen expression in proximal tubular cells, Am. J. Phys. Cell Phys. 304 (7) (2013) C685–C692.
[16] L. Jackson, et al., Within the brain: the renin angiotensin system, Int. J. Mol. Sci. 19 (3) (2018) 876.
[17] A.M. Allen, J. Zhuo, F.A. Mendelsohn, Localization and function of angiotensin AT1 receptors, Am. J. Hypertens. 13 (S1) (2000) 31S–38S.
[18] G.R. Kinsey, L. Li, M.D. Okusa, Inflammation in acute kidney injury, Nephron Exp. Nephrol. 109 (4) (2008) e102–e107.
[19] N. Tsao, et al., Tumour necrosis factor-α causes an increase in blood-brain barrier permeability during sepsis, J. Med. Microbiol. 50 (9) (2001) 812–821.
[20] R. Rodríguez-Romo, et al., AT1 receptor antagonism before ischemia prevents the transition of acute kidney injury to chronic kidney disease, Kidney Int. 89 (2) (2016) 363–373.
[21] S.M. Molinas, et al., Effects of losartan pretreatment in an experimental model of ischemic acute kidney injury, Nephron Exp. Nephrol. 112 (1) (2009) e10–e19.
[22] J.L. Labandeira-Garcia, et al., Brain renin-angiotensin system and microglial

polarization: implications for aging and neurodegeneration, Front. Aging Neurosci. 9 (2017) 129.
[23] W. Jing, B. Jabbari, N.D. Vaziri, Uremia induces upregulation of cerebral tissue oxidative/inflammatory cascade, down-regulation of Nrf2 pathway and disruption of blood brain barrier, Am. J. Transl. Res. 10 (7) (2018) 2137.
[24] K. Fujisaki, et al., Cerebral oxidative stress induces spatial working memory dysfunction in uremic mice: neuroprotective effect of tempol, Nephrol. Dial. Transplant. 29 (3) (2013) 529–538.
[25] M. Horiuchi, M. Mogi, Role of angiotensin II receptor subtype activation in cognitive function and ischaemic brain damage, Br. J. Pharmacol. 163 (6) (2011) 1122–1130.
[26] N. Pelisch, et al., Blockade of AT1 receptors protects the blood–brain barrier and improves cognition in Dahl salt-sensitive hypertensive rats, Am. J. Hypertens. 24 (3) (2011) 362–368.
[27] D.-H. He, et al., Long-term prehypertension treatment with losartan effectively prevents brain damage and stroke in stroke-prone spontaneously hypertensive rats, Int. J. Mol. Med. 33 (2) (2014) 301–309.
[28] M. Palkovits, et al., Neuronal activation in the central nervous system of rats in the initial stage of chronic kidney disease-modulatory effects of losartan and moxonidine, PLoS One 8 (6) (2013) e66543.
[29] G.H. Heeba, Angiotensin II receptor blocker, losartan, ameliorates gentamicin-induced oxidative stress and nephrotoxicity in rats, Pharmacology 87 (3–4) (2011) 232–240.
[30] M. Haghighi, et al., The role of angiotensin II receptor 1 (AT1) blockade in cisplatin-induced nephrotoxicity in rats: gender-related differences, Ren. Fail. 34 (8) (2012) 1046–1051.
[31] M. Malek, M. Nematbakhsh, The preventive effects of diminazene aceturate in renal ischemia/reperfusion injury in male and female rats, Adv. Prev. Med. 2014 (2014).
[32] M. Fang, et al., Circular RNA involved in the protective effect of losartan on ischemia and reperfusion induced acute kidney injury in rat model, Am. J. Transl. Res. 11 (2) (2019) 1129.
[33] P. Williams, et al., Characterization of renal ischemia-reperfusion injury in rats, J. Pharmacol. Toxicol. Methods 37 (1) (1997) 1–7.
[34] S. Soman, et al., Oxidative stress induced NMDA receptor alteration leads to spatial memory deficits in temporal lobe epilepsy: ameliorative effects of Withania somnifera and Withanolide A, Neurochem. Res. 37 (9) (2012) 1915–1927.
[35] E.A. Johnson, A Study of the Effects of Immersion on Short-term Spatial Memory, (2010).
[36] R. D'Hooge, P.P. De Deyn, Applications of the Morris water maze in the study of learning and memory, Brain Res. Rev. 36 (1) (2001) 60–90.
[37] A. Roozbehi, et al., The impact of using different doses of progesterone on memory performance, Bratisl. Lek. Listy 118 (7) (2017) 405–407.
[38] G. Paxinos, K.B. Franklin, Paxinos and Franklin's the Mouse Brain in Stereotaxic Coordinates, Academic press, 2019.
[39] Júnior, H.V.N., M.M.d.F. Fonteles, and R. Mendes de Freitas, Acute seizure activity promotes lipid peroxidation, increased nitrite levels and adaptive pathways against oxidative stress in the frontal cortex and striatum. Oxidative Med. Cell. Longev., 2009. 2(3): p. 130–137.
[40] S.-Y. Cheng, et al., Losartan reduces ensuing chronic kidney disease and mortality after acute kidney injury, Sci. Rep. 6 (2016) 34265.
[41] N. Haruyama, et al., Improvement in spatial memory dysfunction by telmisartan through reduction of brain angiotensin II and oxidative stress in experimental uremic mice, Life Sci. 113 (1–2) (2014) 55–59.
[42] W. Cao, et al., Reno-cerebral reflex activates the renin-angiotensin system, promoting oxidative stress and renal damage after ischemia-reperfusion injury, Antioxid. Redox Signal. 27 (7) (2017) 415–432.
[43] J.M. Saavedra, E. Sánchez-Lemus, J. Benicky, Blockade of brain angiotensin II AT1 receptors ameliorates stress, anxiety, brain inflammation and ischemia: therapeutic implications, Psychoneuroendocrinology 36 (1) (2011) 1–18.
[44] J. Wu, et al., Irbesartan-mediated AT1 receptor blockade attenuates hyposmotic-induced enhancement of I Ks current and prevents shortening of action potential duration in atrial myocytes, J. Renin-Angiotensin-Aldosterone Syst. 15 (4) (2014) 341–347.
[45] S. Heemskerk, et al., Selective iNOS inhibition for the treatment of sepsis-induced acute kidney injury, Nat. Rev. Nephrol. 5 (11) (2009) 629.
[46] P. Garry, et al., The role of the nitric oxide pathway in brain injury and its treatment—from bench to bedside, Exp. Neurol. 263 (2015) 235–243.
[47] A. Nongnuch, K. Panorchan, A. Davenport, Brain–kidney crosstalk, Crit. Care 18 (3) (2014) 225.
[48] D. Armstrong, et al., Angiotensin II blockade of long-term potentiation at the perforant path-granule cell synapse in vitro, Peptides 17 (4) (1996) 689–693.
[49] J.B. Denny, et al., Angiotensin II blocks hippocampal long-term potentiation, Brain Res. 567 (2) (1991) 321–324.
[50] R. Fogari, et al., Influence of losartan and atenolol on memory function in very elderly hypertensive patients, J. Hum. Hypertens. 17 (11) (2003) 781.
[51] J.M. Starr, L.J. Whalley, I.J. Deary, The effects of antihypertensive treatment on cognitive function: results from the HOPE study, J. Am. Geriatr. Soc. 44 (4) (1996) 411–415.
[52] P.G. Kehoe, G.K. Wilcock, Is inhibition of the renin–angiotensin system a new treatment option for Alzheimer's disease? Lancet Neurol. 6 (4) (2007) 373–378.
[53] O. Hanon, et al., Effects of hypertension therapy based on eprosartan on systolic arterial blood pressure and cognitive function: primary results of the Observational Study on Cognitive function And Systolic Blood Pressure Reduction open-label study, J. Hypertens. 26 (8) (2008) 1642–1650.
[54] M.K. Raizada, et al., Increased angiotensin II type-1 receptor gene expression in neuronal cultures from spontaneously hypertensive rats, Endocrinology 132 (4) (1993) 1715–1722.
[55] J. Zhou, et al., Angiotensin II AT1 receptor blockade abolishes brain microvascular inflammation and heat shock protein responses in hypertensive rats, J. Cereb. Blood Flow Metab. 25 (7) (2005) 878–886.
[56] H. Ando, et al., Angiotensin II AT1 receptor blockade reverses pathological hypertrophy and inflammation in brain microvessels of spontaneously hypertensive rats, Stroke 35 (7) (2004) 1726–1731.
[57] H.A. Ahmed, et al., RAS modulation prevents progressive cognitive impairment after experimental stroke: a randomized, blinded preclinical trial, J. Neuroinflammation 15 (1) (2018) 229.
[58] J. Calviño, et al., Long-term anti-proteinuric effect of Losartan in renal transplant recipients treated for hypertension, Nephrol. Dial. Transplant. 15 (1) (2000) 82–86.
[59] Z. Wang, et al., Protective effects of aliskiren on ischemia–reperfusion-induced renal injury in rats, Eur. J. Pharmacol. 718 (1–3) (2013) 160–166.
[60] U. Srisawat, et al., Losartan and sodium nitroprusside effectively protect against renal impairments after ischemia and reperfusion in rats, Biol. Pharm. Bull. 38 (5) (2015) 753–762.
[61] N. Vaziri, et al., Intra-renal angiotensin II/AT1 receptor, oxidative stress, inflammation, and progressive injury in renal mass reduction, J. Pharmacol. Exp. Ther. 323 (1) (2007) 85–93.
[62] R.H. Böger, et al., ADMA and oxidative stress may relate to the progression of renal disease: rationale and design of the VIVALDI study, Vasc. Med. 10 (1_suppl) (2005) S97–S102.
[63] S.D. Crowley, N.P. Rudemiller, Immunologic effects of the renin-angiotensin system, J. Am. Soc. Nephrol. 28 (5) (2017) 1350–1361.
[64] J. Benicky, et al., Angiotensin II AT 1 receptor blockade ameliorates brain inflammation, Neuropsychopharmacology 36 (4) (2011) 857.
[65] C.J. Maxwell, D.B. Hogan, Antihypertensive Agents and Prevention of Dementia, British Medical Journal Publishing Group, 2010.
[66] P.R. Gard, The role of angiotensin II in cognition and behaviour, Eur. J. Pharmacol. 438 (1–2) (2002) 1–14.
[67] V. Raghavendra, K. Chopra, S. Kulkarni, Comparative studies on the memory-enhancing actions of captopril and losartan in mice using inhibitory shock avoidance paradigm, Neuropeptides 35 (1) (2001) 65–69.
[68] M. Kucuk, et al., Effects of losartan on the blood–brain barrier permeability in long-term nitric oxide blockade-induced hypertensive rats, Life Sci. 71 (8) (2002) 937–946.
[69] M. Kaya, et al., Effect of losartan on the blood–brain barrier permeability in diabetic hypertensive rats, Life Sci. 73 (25) (2003) 3235–3244.
[70] N.-C. Li, et al., Use of angiotensin receptor blockers and risk of dementia in a predominantly male population: prospective cohort analysis, Bmj 340 (2010) b5465.
[71] R. Mechaeil, et al., Cognitive enhancement following acute losartan in normotensive young adults, Psychopharmacology 217 (1) (2011) 51–60.