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Angiotensin II and its receptor in activated microglia enhanced neuronal loss and cognitive impairment following pilocarpine-induced status epilepticus

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ABSTRACT

Neuroinflammation plays a role in the pathology of epilepsy and in cognitive impairment. Angiotensin II (AII) and the angiotensin receptor type 1 (AT1) have been shown to regulate seizure susceptibility in different models of epilepsy. Inhibition of AT1 attenuates neuroinflammatory responses in different neurological diseases. In the present study, we showed that the protein expression of AII and AT1 was increased in activated microglia following lithium pilocarpine-induced status epilepticus (SE) in rats. Furthermore, the AT1 receptor antagonist, losartan, significantly inhibited SE-induced cognitive impairment and microglia-mediated inflammation. Losartan also prevented SE induced neuronal loss in the hippocampus and exerted neuroprotection. These data suggest that losartan improves SE-induced cognitive impairment by suppressing microglia mediated inflammatory responses and attenuating hippocampal neuronal loss. Overall, our findings provide a possible therapeutic strategy for the treatment of cognitive impairment in epilepsy.

1. Introduction

Numerous studies have shown a strong link between neuroinflammation and neurodegeneration (Xanthos and Sandkühler, 2014). Neuroinflammation directly influences neuronal function and accelerates neuronal loss after trauma, and both ischemic and excitotoxic brain injuries (J. Chen et al., 2014; Finnie, 2013). Increasing evidence is also pointing to a significant role of neuroinflammation in the pathology of epilepsy, both in seizures and in the formation of pathological lesions after seizures (Xanthos and Sandkühler, 2014; De Araujo Furtado et al., 2012). Hippocampal inflammation has been shown to result in cognitive impairment associated with epilepsy (Avanzini et al., 2013) and microglial-mediated inflammatory responses are likely to be involved in this event (Sierra et al., 2014). We have previously demonstrated that inhibiting astrocyte activation improves cognitive impairment in epilepsy (Sun et al., 2012). However, the specific underlying mechanisms have yet to be understood.

The renin–angiotensin system controls blood pressure. Angiotensin II (AII) plays a major role in the initiation and progression of cardiovascular disease. Recent studies have suggested that AII is a neurotransmitter and neuromodulator (Wright and Harding, 2013). Blocking the AT1 receptor may attenuate inflammation and oxidative stress (Saavedra et al., 2011). Inhibition of AT1 in the rat brain improves the neurological outcome and reduces the infarct volume after experimental ischemia (Saavedra et al., 2011). AII also increases burst discharges and neuronal excitability in the hippocampus via the activation of AT1 (Łukawski et al., 2010). Furthermore, AII and AT1 regulate seizure susceptibility in various models of epilepsy (Tchekalarova and Georgiev, 2005; Pereira et al., 2010). In a model of PTZ kindling, AII receptor antagonists decrease the seizure intensity (Tchekalarova and Georgiev, 2006). Angiotensin-converting-enzyme inhibitors and AII receptor antagonists prevent neuronal loss in the hippocampus during epilepsy, and inhibit neuroinflammatory responses (De Bundel and Smolders, 2008). AT1 blockers have also been shown to activate peroxisome proliferator-activated receptor gamma, exert anti-inflammatory effects, and prevent neuronal loss in CNS (Villapol et al., 2012). Although AII plays a role in seizure susceptibility and neuronal loss, its mechanism of action is still debatable.

Epilepsy is a paroxysmal neurological disorder that is commonly associated with cognitive impairment, with both clinical and non-clinical studies showing that cognitive impairment is exacerbated following a higher frequency of seizures (Vijayaraghavan et al., 2011). Both animal and human studies have suggested that seizure-induced cognitive

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impairment is mediated by the hippocampus (Tchekalarova and Georgiev, 2006), and that cognitive decline is a result of a chronic inflammatory response of the hippocampus (Sun et al., 2012). Clinically, hippocampal sclerosis is associated with neuronal loss and marked gliosis in patients with mesial temporal lobe epilepsy (Bandopadhyay et al., 2014). Inhibition of hippocampal inflammation improves cognitive impairment in various models of epilepsy (Avanzini et al., 2013; Galanopoulou, 2013).

Overall, AII and the AT1 receptor are suggested to be involved in neuroinflammation, neuronal excitability, and neuronal loss in the hippocampus during epilepsy. However, their mechanism of action remains unclear. Furthermore, whether AT1 antagonists affect cognitive function by preventing hippocampal inflammation during epilepsy is still unclear. Therefore, in the present study, we examined the expression of AII and AT1 in the hippocampus. We also investigated the role of the AT1 antagonist, losartan, on microglia-mediated inflammatory effects and cognitive impairment in lithium pilocarpine-induced status epilepticus (SE) in rats.

2. Results

2.1. SE increased the hippocampal levels of AII and AT1

We first investigated the temporal effect of SE on the hippocampal levels of AII and AT1 by the enzyme immunoassay and Western blots, respectively. AII levels peaked 7 days after SE and decreased slightly (but remaining above control levels) 14 days after SE (Fig. 1A). AT1 progressively increased 3, 6 and 24 h, and 3, 7 and 14 days after SE, peaking 3 days after SE. There was statistically significant difference compared with the control group (Fig. 1B and C, $^{**}P < 0.01$, $^{*}P < 0.05$ by ANOVA with Dunnett's post test).

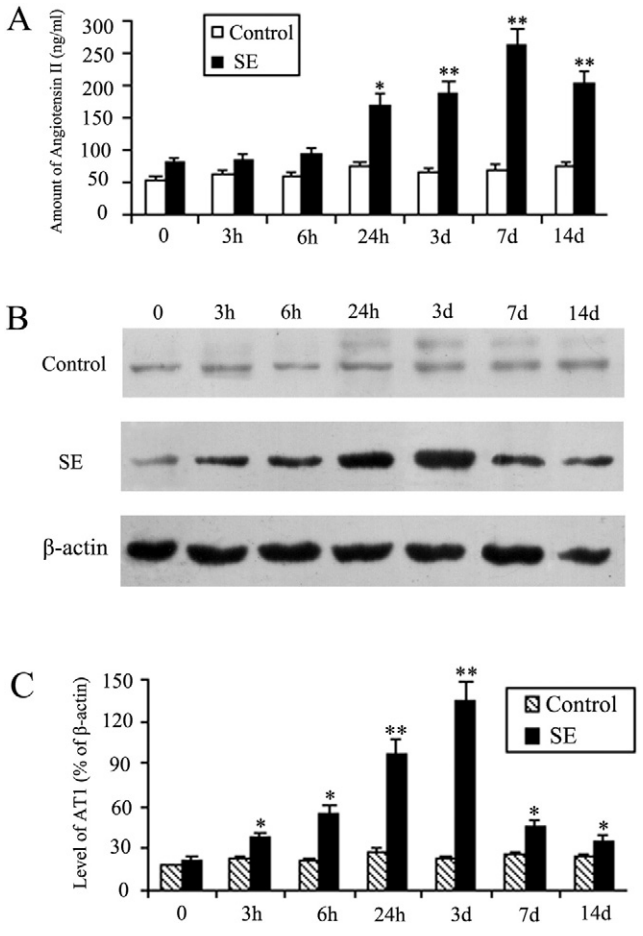

Fig. 1. SE increased the hippocampal levels of AII and AT1. (A) AII concentration in the rat hippocampus samples at 3, 6 and 24 h, and 3, 7 and 14 days after SE, and the control group as determined by EIA. (B–C) Western blot analysis of AT1 concentration at different time points in the hippocampus after SE. Data represent mean ± S.E.M. $^{**}P < 0.01$; $^{*}P < 0.05$ vs. control animals (0 days), (n = 5 per group, ANOVA with Dunnett's post test).

2.2. SE induced the expression of AII and AT1 in activated hippocampal microglia

First, we examined the effect of SE on the localization of AII and AT1 in the CA1 region of the hippocampus. In control rats, AII/AT1 was mainly localized in neurons and not detected in microglia (Fig. 2A). Three days and 1 week after SE, intense immunoreactivity for AII/AT1 was detected in activated microglial cells. Immunofluorescence double staining 7 days after SE demonstrated that AII/AT1 immunoreactive cells were clearly hypertrophic, amoebic, thus morphologically activated microglia (Fig. 2B). Second, the microglia were sorted using fluorescence-activated cell sorting (FACS) and then the expression of AT1 mRNA in microglia was directly determined by RT-PCR. Results confirmed that the CD11b + microglia was the important cellular source of AT1 during experimental epileptic rat (Fig. 3). RT-PCR analysis of AT1 mRNA also confirmed the Western blot results (Fig. 3C).

2.3. Losartan blocked SE-mediated microglial activation in the hippocampus

Losartan, as the AT1 receptor antagonist, was injected intracerebroventricularly 1 h before the injections of lithium chloride, and was administered everyday as described above until every animal was killed. Results showed that losartan reduced the density and activation of microglia in the CA1 regions of the hippocampus. Compared with the vehicle-treated SE group, microglial cells of the losartan group exhibited shorter processes and their cell bodies were smaller (Fig. 4B and C). Quantitative analysis of OX-42 positive cells in the CA1 region of the losartan-treated SE group revealed that these cells were significantly reduced compared with the vehicle-treated SE group ($^{**}P < 0.01$ by ANOVA with Dunnett's post test, Fig. 4E). Although Western blot analysis showed that minocycline significantly inhibited the expression of AT1, there was no statistically significant difference compared with the losartan treated SE group (▲P > 0.05 by ANOVA with Dunnett's post test, Fig. 4F and G).

2.4. Losartan inhibited the SE-mediated expression of hippocampal TNF-α

ELISA analysis showed that a 24 h exposure of SE increased the expression of TNF-α in the hippocampus and losartan inhibited this response (Fig. 5A). Compared with the vehicle-treated SE group, losartan reduced the TNF-α concentration by 66% (pg/mg wet weight tissue: control, 3.43 ± 0.37, vehicle, 34.7 ± 7.3, losartan, 11.8 ± 0.36).

2.5. Losartan did not change AT1 mRNA expression on neurons after SE

To further show that losartan directly inhibited microglia-mediated inflammation, and to determine whether losartan can influence AT1 expression in neurons in the brain injury after SE, we used fluorescence-activated cell sorting of neurons (Thy-1 +) from the hippocampus for the analysis of the AT1 mRNA expression in epileptic rat. As expected, losartan did not change AT1 mRNA expression in neurons after SE (Fig. 5B).

2.6. Losartan inhibited SE-mediated hippocampal neuronal loss

To determine if losartan exerted a neuroprotective effect after SE, we examined the effect of this antagonist on neuronal loss in the CA1, CA3, and hilus regions of the hippocampus 14 days after SE. Compared with