Commentary

Brain angiotensin system: a new promise in the management of epilepsy?

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Epilepsy is a highly prevalent neurological disease and anti-epileptic drugs (AED) are almost the unique clinical treatment option. A disbalanced brain renin–angiotensin system (RAS) has been proposed in epilepsy and several reports have shown that angiotensin II (Ang II) receptor-1 (ATR1) activation is pro-inflammatory and pro-epileptogenic. In agreement, ATR1 blockage with the repurposed drug losartan has shown benefits in animal models of epilepsy. Processing of Ang II by ACE2 enzyme renders Ang-(1-7), a metabolite that activates the mitochondrial assembly (Mas) receptor (MasR) pathway. MasR activation presents beneficial effects, facilitating vasodilatation, increasing anti-inflammatory and antioxidative responses. In a recent paper published in Clinical Science, Gomes and colleagues (Clin. Sci. (Lond.) (2020) 134, 2263–2277) performed intracerebroventricular (icv) infusion of Ang-(1-7) in animals subjected to the pilocarpine model of epilepsy, starting after the first spontaneous motor seizure (SMS). They showed that this approach reduced the frequency of SMS, restored animal anxiety, increased exploration, and augmented the hippocampal expression of protective catalase enzyme and antiapoptotic protein B-cell lymphoma 2 (Bcl-2). Interestingly, but surprisingly, Gomes and colleagues showed that MasR expression and mTor activity were reduced in the hippocampus of the epileptic Ang-(1-7) treated animals. These results show that Ang-(1-7) administration could represent a new avenue for developing strategies for the management of epilepsy in clinical settings. However, future work is necessary to evaluate the levels of RAS metabolites and the activity of key enzymes in these experimental interventions to completely understand the therapeutic potential of the brain RAS manipulation in epilepsy.

Epilepsy is a highly prevalent neurological chronic disorder that presents high heterogeneity when considering etiology, seizures types, and outcome. In spite of extensive research in the last decades, the anti-convulsive therapies with anti-epileptic drugs (AED) prevent the recurrent seizures in most patients, but do not significantly affect the history of the disease in a number of patients that finally develop AED-resistant epilepsy [1–3]. Approximately 30% of the patients never reach a sustained remission from seizures with AED therapies [1–4], suffering from refractory epilepsy that has serious social and physical consequences such as progressive cognitive impairment, alterations in behavior and depression [5,6]. Resistance to AED seems not to be just a consequence of drugs long-term use. A number of studies have shown that several predictors are associated with resistance to AED including patients age, sex, type of epilepsy, onset age, number of seizures experienced before treatment, electrical and/or imaging evidence of neurological insults, and familiar story of epilepsy [1,4,7]. It is also clear that personalized approaches involving strict therapeutic drug monitoring and pharmacogenetics studies represent substantive advantages for these patients [8].
Figure 1. Schematic representation of brain RAS main features

(A) The figure shows the members of the neurovascular unit [vascular endothelium, astrocytes (A), microglia (M) and neurons (N)] and summarizes the receptors that have been described in each cell type of the neurovascular unit in the CNS. While there is some degree of controversy about the presence of AT1R and ATR2 in glial cells in a non-injured brain, there is stronger evidence that ATR are expressed in glial cells after brain injury, and that angiotensin derivates have effects on glial cells, being specifically ATR1 a pro-inflammatory inducer and ATR2/MasR pathways anti-inflammatory. Activation of the MasR downstream signaling by applying Ang-(1-7) is probably beneficial due to anti-inflammatory mediators released by glial cells; by direct activity on neuronal MasR and also by modifying the RAS equilibrium in the epileptic brain. (B) Scheme on the proposed interactions among the members of brain RAS and their effect in the central nervous system (CNS).

Temporal lobe epilepsy (TLE) is the most common human epilepsy and retrospective studies have shown that a significant number of patients refer an early precipitating event during childhood, followed by a silent period of several years until the chronic stage of the disease (see as example [9]). The silent phase of the disease is a poorly studied period when epileptogenesis is proposed to occur and thus it could be a period of utmost importance to prevent the development of the chronic phase of the disease. Studies based on animal models of epilepsy have shown a proof-of-concept for this window of intervention [10,11]. Once the chronic disease is installed and spontaneous seizures emerge, the AED therapies are the clinical treatment first choice.

The recent article published in Clinical Science by Gomes and colleagues (2020) [12] explores the modulation of the brain renin–angiotensin system (RAS) as a potential target to intervene when the chronic seizures are installed. By performing a series of studies in rats exposed to the pilocarpine model of epilepsy that developed spontaneous motor seizures (SMS), the authors chronically administered angiotensin-(1-7) [Ang-(1-7)] and showed a significative reduction in the SMS, an improvement in several behavioral parameters, as well as increased hippocampal levels of protective catalase enzyme and anti-apoptotic B-cell lymphoma 2 (Bcl-2) [12].

Originally described in the control of cardiovascular function, the role of the brain RAS was unveiled in the last decade. Several organs, including the brain, have the ability to synthesize angiotensinogen which is the precursor of all angiotensin metabolites. Multiple studies have shown that brain angiotensinogen levels are independent of circulating angiotensinogen levels thus reinforcing the idea that brain has its own production of RAS metabolites [13]. Furthermore, angiotensinogen- and angiotensin II (Ang II)-positive cells in the brain were identified and RAS components are present in the brain [14–16]. Studies suggested that astrocytes are the main source of angiotensinogen and they also express angiotensin receptor-1 (ATR1) and mitochondrial assembly receptor (MasR); while neurons express ATR1, angiotensin-converting enzyme-2 (ACE2), MasR; and microglia respond to ATR1 [16]. Additionally, the brain shows high MasR expression and also high Ang-(1-7) concentrations have been detected in this organ [17]. On the other hand, ACE2 is expressed in brain endothelium, neurons and probably in glial cells [15,16,18,19] (Figure 1A).

Conversion of angiotensinogen into angiotensin I and subsequently into Ang II by ACE1 allows different responses in the brain. Ang II binds to ATR1 and promotes vascular contractility, inflammation and oxidative stress; while
Ang-(1-7), produced by ACE2, activates MasR and counteracts the effects of the Ang II/AT1R axis (see revision in [20]). Counteracting the Ang II/ATR1 effects, the activation of brain Ang-(1-7)/MasR pathway produces beneficial effects for neuronal survival such as vasodilatation, anti-inflammation, antioxidant, and activates anti-apoptotic pathways (reviewed in [21,22,23,24]; Figure 1B). The role of RAS in neuroinflammation, and specifically the balance of ATR1/MasR, is critical. For example, microglial ATR1 stimulation activates NF-κB and subsequent release of pro-inflammatory cytokines [16] that expand neuroinflammation, recruit immune cells and activate astrocytes. Overall, it is clear that, having Ang II and Ang-(1-7) opposed effects, the role of ACE2 in the maintenance of brain RAS equilibrium is critical in healthy and diseased brains. As a side note, the prominent role of ACE2 as viral spike receptor in the present SARS-CoV-2 pandemic has raised a number of hypotheses concerning the potential role of RAS in the neuroinflammation and neurological alterations in COVID-19 affected patients. Present hypotheses propose that virus entrance to cells dramatically depletes ACE2 and thus decreases beneficial Ang-(1-7)/MasR activity and increases the detrimental Ang II/ATR1 pathway in different organs, including the brain (see as examples [21,22,25]; Figure 1B).

The disbalance in the brain RAS has been described in epilepsy and, specifically the hyperactivity of the Ang II/ATR1 pathway, has been repeatedly reported. An early report from Argañaraz and colleagues (2008) [26] showed that TLE patients with mesial temporal sclerosis have up-regulation of AT1R receptor in the cortex and hippocampus. In an experimental epilepsy model in rats it was shown that some components of brain RAS (ACE1 and AT1R) were up-regulated and the use of ACE1 inhibitor enalapril, or AT1R antagonist losartan, reduced seizures [27]. Further studies in the pilocarpine model of epilepsy showed a biphasic effect, with a predominant expression of Ang-(1-7) in the acute and silent period, and a decrease in the chronic phase accompanied of increased Ang II/AT1R [28,29].

Reinforcing the concept of a detrimental role of ATR1 activation and the importance of the control of brain RAS in epilepsy, Ivanova and Tchekalarova [30] demonstrated that administration of Ang II during the silent phase of epilepsy following kainate-induced status epilepticus is pro-epileptogenic decreasing the latency for onset of the first spontaneous seizure, increased the frequency of seizures and exacerbated the kainate-induced hyperactivity, but surprisingly improving neuronal survival [30]. Systemic or intracerebroventricular treatment with ATR1 antagonist losartan also increased the number of stimulations required to reach the fully kindled state in a model of amygdala kindling, however without having effects in the threshold or seizures susceptibility [31].

Using resected tissue from patients with pharmacoresistant TLE, Reyes-Garcia and colleagues [32] reported that losartan did not exert effects on epileptiform activity induced by high potassium concentration in human brain slices. This fact is an important warning sign pointing out the reduced efficacy of AT1R blocker losartan preventing acute epileptiform activity, at least in the in vitro setting [32]. It is important, however, to consider that several animal studies have shown positive effects of losartan during the latency period besides antiepileptic effects [33,34]. Additionally, losartan in vivo is probably modulating the brain RAS balance, for example increasing the availability of free Ang II to be converted into beneficial Ang-(1-7) if an adequate ACE2 activity level is present.

In this scenario, the beneficial effects of Ang-(1-7) reported in the recent paper by Gomes and colleagues [12] are likely bypassing the need of ATR1 blockage to directly increase Ang-(1-7) level and thus expanding its beneficial effects. Moreover, it is interesting to put these findings [12] into the context of the reported evidence showing down-regulation of Ang-(1-7) in the chronic phase of epilepsy [28] since the administration of Ang-(1-7) probably restores the levels of this beneficial mediator in the brain parenchyma, thus preventing further detrimental effects of chronic seizures and probably the progression to more severe brain tissue damage. This hypothesis may itself justify further investigations on the potential modulation of this pathway in AED-resistant epilepsy where only very limited options can be offered to patients.

Controlling epileptogenesis before the manifestation of epilepsy as chronic disease is another interesting research avenue for new therapeutic strategies aimed to change natural story of epilepsy. Brain RAS therapeutic modulation also seems to be beneficial during the silent phase of epilepsy. ATR1 blockage with losartan increased the duration of seizure-free period, decreased the frequency of SMS and protected hippocampal CA1 neurons in a model of kainate-induced epileptogenesis [33]. As stated above, at least part of the protective effects achieved by ATR1 blocker losartan in the lithium-pilocarpine model of epilepsy have been attributed to the modulation of astroglial AQP4 expression [34] and to the reduction in microglial pro-inflammatory response [29]. It is clear that glial cells seem to have a main role during this silent epileptogenic period [10,11] and, concomitantly, hyperactivation of Ang II/ATR1 signaling has been observed in astrocytes and microglia [31,30].

Initial findings justified the beneficial effects of modulating brain RAS in epilepsy by the blockage of the serum albumin/TGFβ effects after blood–brain barrier breakdown [35]. However, as summarized in this short article, a growing body of data show that brain RAS modulation, including ATR1 blockage and/or increasing the activity of
the Ang-(1-7)/MasR pathway by directly administrating Ang-(1-7), has a wider action exerting a plethora of beneficial effects, both during the silent phase of epilepsy and also when the spontaneous chronic seizures are established.

The brain RAS was proposed to be independent from the cardiovascular–renal RAS due to the isolation provided by the blood–brain barrier, however spontaneously hypertensive rats (SHRs) subjected to kainate-induced epilepsy paradigm showed increased AT1R hippocampal expression compared with normotensive rats and the AT1R antagonist losartan reduced AT1R expression with stronger effects in SHR compared with wild type rats [36]. These findings underline the importance of further studies to understand the cross-talk between brain and periphery RAS as well as the physiopathological basis of the comorbidity of hypertension and epilepsy in humans.

In summary, brain RAS modulation represents a novel and exciting candidate pathway to develop new treatment strategies, not only for chronic or AED-resistant epilepsy, but also to interfere with natural story of the disease by decreasing epileptogenesis. However, the failure to demonstrate in vitro effects of ATR1 blockage in human epileptic tissue [32] should drive our attention to the potential requirement of a living system with an active neurovascular unit to achieve beneficial effects of the brain RAS modulation. The interaction among endothelial cells, glia and neurons seems to be required to get beneficial effects by modifying the balance among the different brain RAS ligands and receptors (Figure 1). The beneficial effects reported by Gomes and colleagues [12] are probably the resultant of additive effects of Ang-(1-7) on the MasR pathway as well as a modification of the brain RAS balance affecting, not only neurons, but also endothelium and glial cells. The intriguing reduction in MasR expression observed by the authors could be indicative of such modifications in the entire brain RAS balance, added to the fact that a report showed that Ang-(1-7) effects could be due to ATR1 blockage [37]. Expanding our comprehension of the short- and long-term changes in brain RAS equilibrium, specifically angiotensin metabolites level and activity of key enzymes such as renin, ACE1 and ACE2, is mandatory in this scenario. Furthermore, the role of direct conversion of Ang (1-7) from Ang I by endopeptidases, known to be increased in epilepsy models, has been proposed [27, 28]. Lastly, but not less important, is to dissect the role of endothelium, astrocytes, microglia and neurons in the RAS alterations observed in epileptic brains. Receptors and members of the brain RAS have been described in the different cell types of the CNS in normal and hypertensive rodents, however the expression of RAS components and the effects of the RAS activation in each cell type in epilepsy are poorly known. Knowing the consequences of pharmacological intervention on endogenous levels of individual members of the brain RAS, and the effects in the different cell types, will allow us to improve our ability to control, not only seizures in chronically affected and AED-resistant epileptic patients, but also to develop new approaches to prevent epileptogenesis.

Competing Interests
The author declares that there are no competing interests associated with the manuscript.

Abbreviations
AED, anti-epileptic drug; Ang II, angiotensin II; ATR1, Ang II receptor-1; MasR, mitochondrial assembly receptor; RAS, renin–angiotensin system; SMS, spontaneous motor seizure; TLE, temporal lobe epilepsy.

References