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Letter to the Editor

Losartan fails to suppress epileptiform activity in brain slices from resected tissues of patients with drug resistant epilepsy

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Dear Editor,

One-third of patients with temporal lobe epilepsy fail to obtain adequate seizure control despite of the large therapeutic armamentarium of antiepileptic drugs (AEDs) currently available. New therapeutic approaches are required and drugs generally used for non-epileptic disorders, such as rapamycin, everolimus, celecoxib, bumetanide, amiloride, resveratrol and losartan have been investigated in both clinical trials and animal models of epilepsy with favorable outcomes [1]. Losartan, an angiotensin II type 1 receptor (AT1) antagonist, has emerged as a potential anticonvulsant and antiepileptogenic agent in animal models. It has been suggested that losartan has an antiepileptogenic role preventing acquired epilepsy associated with vascular injury in animal models of post-lesional epilepsy by blocking the transforming growth factor beta signaling pathway induced by serum-derived albumin after blood brain barrier breakdown [2]. In this line, the antiepileptogenic and neuroprotective effects of losartan have been reported following kainic acid-induced *status epilepticus* as well as in an animal model of comorbid hypertension and epilepsy [3,4]. The anticonvulsant effects of the AT1 receptor antagonist were shown by the action of losartan in reducing seizure severity in pentylenetetrazol-induced kindling seizures in mice and in an acoustic model of epilepsy [5,6]. Our laboratory described AT1 receptor upregulation in cortical and hippocampal tissues resected from patients with pharmacoresistant mesial temporal lobe epilepsy, suggesting a relation between this type of epilepsy and renin-angiotensin system [7]. Using electrophysiological studies *in vitro*, this study aimed to evaluate the effects of losartan on seizure-like activity in cortical and hippocampal areas of human brain slices of patients with drug resistant temporal lobe epilepsy.

1. Methods

1.1. Subjects

Eight human hippocampal specimens surgically resected from patients with the diagnosis of pharmacoresistant temporal lobe epilepsy were included into the study. Clinical data from patients enrolled were as follows: female 85%; age of epilepsy onset was 13.6 ± 3.9 years; epilepsy duration (years) 24.3 ± 14.2; focal aware seizure per month 4.6 ± 2.9; focal impaired awareness seizure per month 5.0 ± 2.6; generalized seizures per year 0.3 ± 0.4; number of AEDs at the moment of surgery 5.3 ± 1.6; type of hippocampal sclerosis (ILAE classification) type 1 (88%) (see supplementary material for details). All patients were presurgically evaluated in the Epilepsy Treatment Unit at São Paulo Hospital (UNIPETE), in Brazil. Informed consent was obtained from all patients before surgical treatment. The study was approved by the Ethics Committee of Universidade Federal de São Paulo (CAAE 47551015.1.0000.5505).

1.2. Electrophysiological recordings and induction of epileptiform activity

After the preparation of the tissue (see details in the supplementary material), the recordings started 4 h after the slice preparation to allow optimal recovery of the brain tissue after surgery. Extracellular recordings were performed in the granule cell layer of the dentate gyrus and in deep layers V–VI of the temporal neocortex, using glass electrodes (filled with 154 mM NaCl, tip diameter 2–3 μm, resistance 5 to 10 MΩ), placed 150 μm below the surface of the slice.

In order to assess slice viability, bipolar stimulation electrodes (20 μm diameter, platinum wires, tip separation 60–100 μm) were positioned at the CA4/CA3 border in the hippocampus and below layer VI in the white matter of temporal neocortex, then populations spike (PS) amplitudes were obtained at the beginning and at the end of each experiment. Slices with PS amplitude decrease greater than 20% at the end of the experiments were disqualified from the analysis, ensuring the adequate viability of the slices.

Recordings were performed in three epochs; (1) induction of seizure-like events (2) losartan evaluation effect, (3) washout; each one with a duration of 20–25 min. Event rates, durations and amplitudes of events were measured during the last 5 min of each experimental epoch. Control values were determined in other set of experiments as the mean of induction epoch (*e.g.* 20 min) and washout recording (*e.g.* 60 min) on slices in which losartan was not applied. Effect of losartan was normalized and expressed as the percentage of the control value kept after perfusion of drug [(Losartan value/control value) × 100]. The experimental and control values were analyzed using the Spike2 software and custom-made scripts (MATLAB R2016b). The epileptiform

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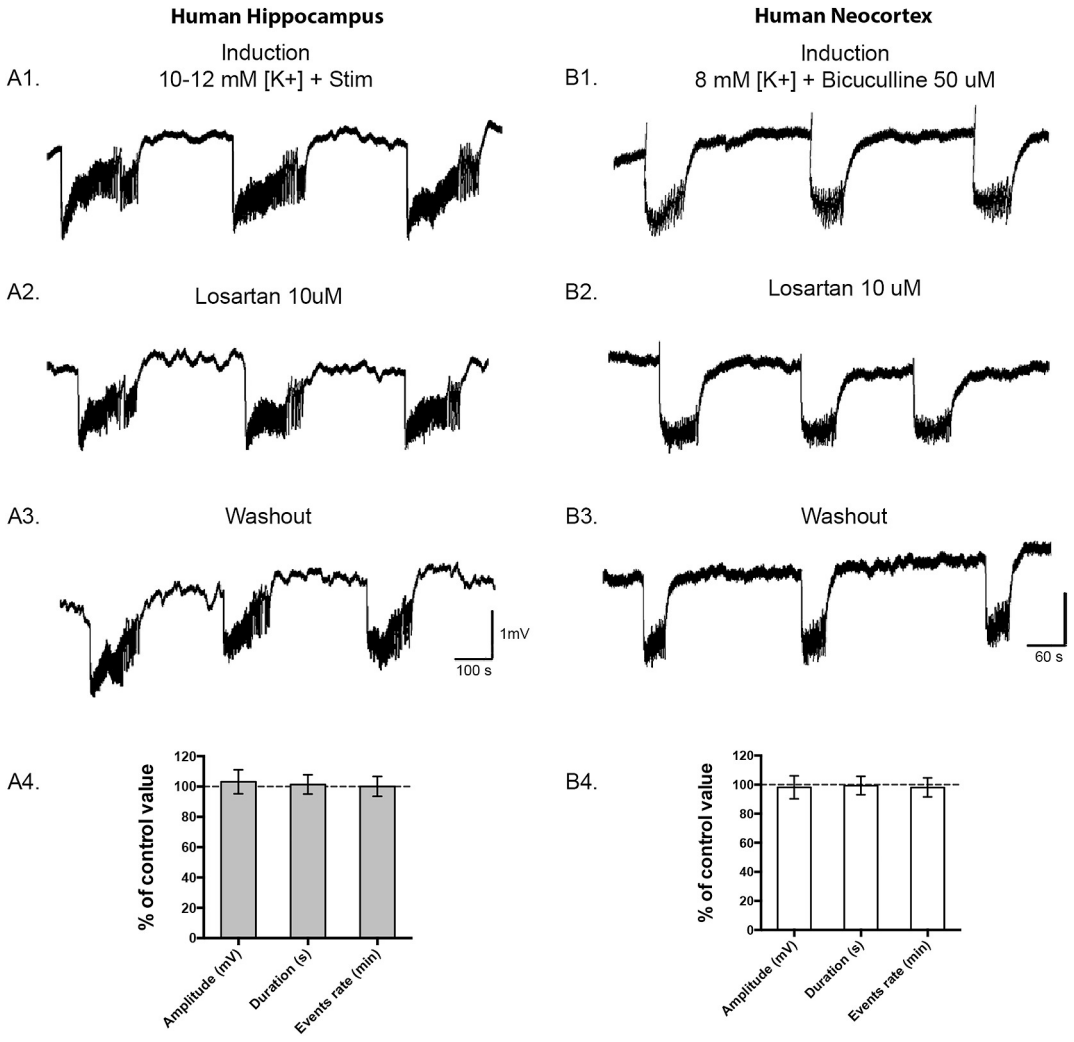

Fig. 1. Effect of losartan on seizure-like events induced in human brain slices (A1-A3) Experimental epochs of epileptifom activity induced in human hippocampus (slices/specimens, 17/8). (B1-B3) Epileptiform activity induced in temporal neocortex (slices/specimens, 18/8). Time and amplitude of ictaform events are given by calibration bars on the bottom. (A4, B4) Effect of losartan on SLEs in each tissue assessed, data are represented as mean ± standard deviation for remaining percentages of control values.

activity was induced in the dentate gyrus by hilar stimulation and continuous perfusion with ACSF containing 10–12 mM of potassium concentration [K+] (high K-ACSF). In the temporal neocortex, high K-ACSF containing 8 mM + 50 μM bicuculline–methiodide were sufficient to provoke epileptiform activity.

2. Results

Seizure-like events were induced in 35 human brain slices (17 hippocampal; 18 cortical; specimens of 8 patients). Nevertheless, losartan (10 and 50 μM) did not exert significant changes in seizure-like activity in both hippocampal and cortical slices. During 25-min of losartan application, the mean values of amplitude, duration and rate of events remained in a range of 94–110% of the control values in the hippocampal slices and 91–105% in the neocortical slices indicating that losartan was unable to reduce or suppress seizure activity (Fig. 1). Indeed, when losartan concentration was increased to 50 μM, spreading depression-like events were induced. In addition to these experiments, a subset of neocortical slices (12 slices from 4 cortical specimens) was used to test whether pre-bathing human slices with ACSF + losartan (during 30 min) could affect the induction of epileptiform activity; however, no changes were observed (see Fig. S1 in supplementary material).

3. Discussion

Our results suggested that losartan did not exert effect on epileptiform activity induced by high potassium concentration in human brain slices from patients with pharmacoresistant temporal lobe epilepsy. The underlying mechanisms involved in seizure generation as used in the *in vitro* experimental approach of our study may be among the possible reasons that could lead to the lack of anticonvulsant efficacy of losartan added to the particularly heterogeneous features (duration of epilepsy, initial precipitant injury, age of onset, antiepileptic drugs used, genetic, among others) of human tissue resected from patients with epilepsy. It is important to point out that is difficult to extrapolate these findings to *in vivo* studies in which the underlying mechanisms of the losartan effects could be influenced by multiple signaling pathways. In this regard, other authors have investigated the anticonvulsant action of losartan in different scenarios using *in vivo* approaches. Georgiev et al., demonstrated that losartan administered intracerebroventricularly tended to decrease seizure intensity in pentylenetetrazol kindling seizures in mice [5]. Additionally, Pereira et al., in an animal model of acoustic epilepsy, showed reduction in tonic clonic seizures in animals treated with losartan [6]. In turn, Łukawski et al., using a model of maximal electroshock in mice, reported that losartan enhances the anticonvulsant activity of lamotrigine [8], gabapentine [9] and valproate magnesium

[10]. These studies as well as a previous work of our laboratory [7] have suggested the role of the renin angiotensin system in epilepsy however our findings do not support the anticonvulsant role of losartan *in vitro*.

4. Conclusion

Despite our observation of the lack of efficacy of losartan in suppressing human epileptiform activity *in vitro*, the promising antiepileptic role of losartan should not be discarded, and further investigations must be conducted to better understand the effect of this drug in human epilepsy.

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Conflict of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2019.01.008.

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