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# Long-term intracerebroventricular infusion of angiotensin II after kainate-induced status epilepticus: Effects on epileptogenesis, brain damage, and diurnal behavioral changes

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ABSTRACT

Our previous studies revealed that Angiotensin (Ang) II has anticonvulsant effects in acute seizure models. However, data on its role in experimental models of epilepsy are missing. In the present study, we tested whether posttreatment with Ang II after kainate (KA)-induced status epilepticus (SE) can affect epileptogenesis, concomitant behavioral changes, and brain damage. The Wistar rats were intracerebroventricularly infused via osmotic mini-pumps with Ang II (1.52 μg/μl/day for 28 days) after SE. Spontaneous motor seizures (SMS) were video-recorded for up to three months. Locomotor activity, anxiety, and depression-like behavior were evaluated during the last week of drug infusion, while spatial memory was assessed during the 3rd month after SE. Angiotensin II decreased the latency for onset of the first SMS and increased the frequency of SMS two months after SE. The continuous peptide infusion exacerbated the KA-induced hyperactivity and caused depression-like behavior. The reduced anxiety of KA-treated rats was alleviated by Ang II exposure. The KA-induced deficit in the hippocampal-dependent spatial memory was not influenced by Ang II. However, Ang II partially prevented the neuronal damage in the hippocampus, specifically in the CA1 area. The role of $AT_1$ and $AT_2$ receptor activation in the effects of the octapeptide is discussed.



## 1. Introduction

The renin–angiotensin system (RAS) is known to participate in the control of numerous physiological and behavioral functions including regulation of blood pressure, release of pituitary gland hormones, water and salt homeostasis, stress responses, cognitive processes, and depression [1]. Most of these functions are mediated by angiotensin (Ang) II type 1 ($AT_1$) receptors [2–4]. Angiotensin II has been considered as an active ligand at this receptor subtype. High $AT_1$ receptor expression has been reported in brain areas involved in autonomic, hormonal, cerebrovascular, and behavioral regulations, such as the pituitary gland, area postrema, hypothalamus, circumventricular organs (CVOs), and amygdala [5,6]. Besides the well-known classical physiological functions, literature data suggest that $AT_1$ receptor subtype is involved in the modulation of brain excitability, long-term potentiation, and control of seizure susceptibility [7–10]. In this context, our studies [8] and that of Stragier et al. [11] have demonstrated that the biologically active Ang peptides, Ang II, Ang III, and Ang IV, exert an anticonvulsant activity in acute seizure models in rodents, as well as in pentylenetetrazol-, bicuculline-, picrotoxine-, as well as pilocarpine-induced seizures. Moreover, recently presented clinical data, which revealed an upregulation of $AT_1$ receptors and their mRNA expression in the cortex and hippocampus in both patients, diagnosed with temporal lobe epilepsy (TLE) [12], support the presumption that the effects of Ang II on seizure susceptibility might be mediated by the $AT_1$ receptor subtype. Repetitive seizures induced in an experimental model of TLE caused an upregulation of the components of RAS, ACE (angiotensin-converting enzyme), and the $AT_1$ receptor expression in the hippocampus of Wistar audiogenic rats [13] and the pilocarpine model of TLE [14]. The long-term treatment with losartan after kainate (KA)-induced status epilepticus (SE) has been found to increase the latency for onset of the first spontaneous seizure and to prevent some of the deleterious consequences accompanying the chronic epileptic state in rats [15].

In view of the fact that, recently, we have found that acute injection of Ang II possesses an anticonvulsant effect in a battery of seizure tests with different mechanisms of action in mice, in the present study, we aimed further to explore the efficacy of long-term intracerebroventricular

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(i.c.v.) infusion of Ang II, started after KA-induced SE, on the development of epileptogenesis, deleterious behavioral consequences, and brain damage in Wistar rats.

## 2. Material and methods

The procedures used in this study were in agreement with the European Communities Council Directive 2010/63/EU. The experimental design was approved by the Institutional Ethics Committee at the Institute of Neurobiology and the Ethics Committees for research at the Sofia Medical University under the contract No. 30/2011 for the application grant DTK 02/56 2009–2012.

### 2.1. Subjects

The experiments were performed on male Wistar rats (sixty-day old) (250–300 g) obtained from an animal breeding facility of the Institute of Neurobiology, Bulgarian Academy of Sciences. Following arrival in the laboratory, the animals were individually housed under standardized conditions (20 ± 3 °C, 40–50% humidity; 12/12-h light/dark cycle with lights on at 08:00 h) and habituated for 10 days. Food and water were available ad libitum throughout the study except during test procedures.

### 2.2. Experimental design

The rats were randomly distributed in four experimental groups (n = 10–14) as follows: Group I: control sham rats treated with vehicle (C-sham); Group II: control rats treated with Ang II (C-Ang); Group III: sham rats treated with KA (KA–sham); and Group IV: rats treated with KA + Ang II (KA–Ang). The observers, who counted and scored seizures, as well as behavior in these animals, were not aware of the group to which the animals belonged.

### 2.3. Induction of status epilepticus with kainic acid

Twenty-eight rats in total were subjected to KA injection. Kainic acid was diluted in sterile saline (0.9% NaCl) at 2.5 mg/ml. The protocol of KA-induced SE was executed according to [15]. In brief, SE was induced by repetitive injections of KA (Abcam, UK) starting with a dose of 5 mg/kg, i.p. (1 ml/kg) at the first hour of observation. Thereafter, KA was delivered in half of the abovementioned dose every half an hour. Matched controls were treated with an equivalent volume and number of injections of saline. Seizure intensity was evaluated by a modified Racine's scale [16] as previously [17,18]. Only rats which developed SE (i.e., recurrent seizures with bilateral forelimb clonus for at least 3 h) and survived thereafter were included in the subsequent analyses.

### 2.4. Implantation of osmotic mini-pumps

The surgery was performed under ketamine (40 mg/kg) and xylazine (20 mg/kg, intraperitoneally i.p.) anesthesia five days after SE. Following local anesthesia with procaine 0.5% and fixation on a stereotaxic device (Narishige Sci. Inst. Labs, Japan), a midline incision over the skull was made, and the skin and periosteum were removed with aseptic precautions. A 28-gauge, stainless steel cannula (Alzet Brain Infusion Kit 2, Durect, Cupertino, CA) was implanted into the right lateral cerebral ventricle and fixed on the skull with dental cement. The cannula was placed 1.0 mm posterior and 1.4 mm lateral to the bregma. The lower end of the cannula was at a depth of 3.0 mm from the skull, and the upper end was connected to an osmotic mini-pump (Alzet model 2004) for chronic i.c.v. infusion at a pumping rate of 0.23 ± 0.02 μl/h for a 28-day period (model 2004). The pumps were filled with either Ang II (groups II and IV) (Sigma-Aldrich, Bulgaria) (1.52 μg/μl/day) or 0.9% saline solution (groups I and III) and placed subcutaneously on the back of the rats.

### 2.5. Video-monitoring of spontaneous recurrent seizures

Video-monitoring (24 h/day for 12 weeks starting 24 h after SE) was executed by an infrared-sensitive color camera (S-2016, AVTECH, Taiwan, no. AVC307R) connected to a computer. The recordings were visually analyzed by two independent observers for the detection of spontaneous motor seizures (SMS) of class IV or V (secondarily generalized seizures). Partial seizures of classes I and II were neglected because, without simultaneous EEG recording, they could easily be missed. All spontaneous seizures detected during the experimental manipulations when the animals were outside of their boxes were also noted. Several seizure parameters were evaluated: latent seizure-free period and frequency of SMS.

### 2.6. Behavioral tests

The behavioral tests were performed during the last week of Ang II infusion, i.e., between the 25th and the 33rd day after SE. The radial arm maze test was executed between the 10th and the 12th week after SE. The open field (OF) test, elevated plus maze (EPM) test, and forced swim test (FST) were performed at two time points 6 h after lights on/off (at 15:00 p.m. and 03:00 a.m., respectively) under artificial diffused light during the light phase and in red dim light during the dark phase. The behavioral experiments were conducted in a soundproof room, where the animals were moved at least 30 min before each test. The rats that exhibited SMS at least 1 h before starting the test were excluded from the experimental procedure. The behavior in the OF and EPM tests was recorded using an infrared sensitive CCD camera and a video tracking system (SMART PanLab software, Harvard Apparatus, USA).

#### 2.6.1. Open field test

The apparatus consisted of a gray polystyrene box (100 × 100 × 60 cm) divided into two zones: outer square (periphery) and inner square (center). The rat was placed in the center of the box and was allowed to explore it for 5 min. The calculated standard measures were as follows: 1) total distance traveled (cm); 2) time spent in the central zone (sec) vs total time in %; and 3) an anxiety index calculated using the following equation: Anxiety index = 1 − [(Center time / Total time + (Center distance / Total distance)) / 2]. Anxiety index values range from 0 to 1, with a higher value indicating increased anxiety [19]. After each test, the OF was thoroughly cleaned with 0.1% acetic acid solution to prevent any odor traces.

#### 2.6.2. Elevated plus maze test

The apparatus consisted of two open arms (50 × 10 cm), two enclosed arms (50 × 10 × 50 cm), and a central platform (10 × 10 cm) elevated 50 cm above the floor level. At the beginning of the test, the rat was placed on the central platform facing an open arm. The test lasted 5 min. The calculated standard measures were as follows: 1) total distance traveled (cm); 2) time (sec) spent in the open arms vs total time in %; and 3) anxiety index, which unifies all EPM parameters into one ratio. Anxiety index = 1 − [(Open arms time / Total time) + (Distance open arms / Total distance) / 2]. After each test, the EPM was cleaned with 0.1% acetic acid solution.

#### 2.6.3. Sucrose preference test

The test was performed as described previously [18]. Taste preference was expressed as a percentage of the volume of sucrose solution of the total volume of fluid (sucrose plus regular water) consumed during 12 h (light phase — 8:00–20:00 h and dark phase — 20:00–8:00 h).

#### 2.6.4. Forced swim test

The despair-like behavior was evaluated by a classic forced swim test [20]. The test was carried out in a clear and transparent cylinder (50 cm tall–25 cm diameter) filled to a level of 30 cm from the bottom