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Uric acid is released in the brain during seizure activity and increases severity of seizures in a mouse model for acute limbic seizures

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ABSTRACT

Recent evidence points at an important role of endogenous cell-damage induced pro-inflammatory molecules in the generation of epileptic seizures. Uric acid, under the form of monosodium urate crystals, has shown to have pro-inflammatory properties in the body, but less is known about its role in seizure generation. This study aimed to unravel the contribution of uric acid to seizure generation in a mouse model for acute limbic seizures. We measured extracellular levels of uric acid in the brain and modulated them using complementary pharmacological and genetic tools. Local extracellular uric acid levels increased three to four times during acute limbic seizures and peaked between 50 and 100 min after kainic acid infusion. Manipulating uric acid levels through administration of allopurinol or knock-out of urate oxidase significantly altered the number of generalized seizures, decreasing and increasing them by a twofold respectively. Taken together, our results consistently show that uric acid is released during limbic seizures and suggest that uric acid facilitates seizure generalization.

1. Introduction

Epilepsy is a disabling neurological disorder that affects up to 65 million people worldwide and is characterized by spontaneous and recurrent occurrence of seizures (Thurman et al., 2011). Although epileptic seizures are generally thought to be caused by an increased state of brain excitability, it is much less clear what is causing this hyperexcitability (Devinsky et al., 2013). Danger associated molecular patterns (DAMPs) are normal cell constituents that are released into the extracellular milieu during cellular stress or damage and subsequently activate the immune system (Matzinger, 1994). Recently, DAMPs have been shown to be able to increase excitability in the brain (Maroso et al., 2010). Uric acid is a purine degradation product that has multiple functions in the body (Fang et al., 2013). Soluble uric acid is well known for its anti-oxidant capacity, but its crystallized form is known to act as a DAMP in several disorders, such as gout, asthma and liver injury (Kono et al., 2010; Alvarez-Lario and Macarron-Vicente, 2011; Kool et al., 2011; Rock et al., 2013). There are some indications that uric acid might play a role as a DAMP in epilepsy as well. In epilepsy patients, uric acid levels acutely increase upon generalized seizures (Warren et al., 1975; Luhdorf et al., 1978; Stover et al., 1997) and can be chronically elevated when untreated (Hamed et al., 2004, 2007). Uric acid was also reported to increase in the brain in two animal models, 100 min (Layton et al., 1998) and one week (Beal et al., 1991) after kainic acid administration. Furthermore, several components of the inflammatory signaling pathway downstream of uric acid have been shown to affect seizure characteristics in animal models for epilepsy, i.e. caspase-1 (Vezzani et al., 2010) and NLRP3 (Meng et al., 2014). Notably, this pathway leads to the activation of IL-1β, a central pro-inflammatory cytokine involved in excitability (Vezzani et al., 2011). Finally, a number of studies suggest that allopurinol, an inhibitor of uric acid production, is effective as anticonvulsive therapy (Wada et al., 1992; Zagnoni et al., 1994; Murashima et al., 1998; Togha et al.,

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2007), indicating that uric acid is not just an epiphenomenon but might actively contribute to the underlying pathophysiology.

In this study we investigated the role of uric acid in the generation of limbic seizures in a intrahippocampal KA mouse model. To do this, hippocampal uric acid levels were monitored during seizures using microdialysis and the effect of pharmacologically and genetically manipulating uric acid levels on their severity was investigated.

2. Material and methods

2.1. Chemicals and solutions

Modified Ringer's solution, the perfusion fluid used for microdialysis, consisted of 147 mM NaCl, 2.3 mM $CaCl_2$ and 4 mM KCl (all Sigma Aldrich, Schnelldorf, Germany) in ultrapure water and was kept at 4 °C (one week shelf life). KA (R&D systems, Abington, United Kingdom) was dissolved in modified Ringer's solution at a concentration of 4 mg/ml and kept in aliquots at 20 °C. It was diluted further with modified Ringer's solution to a concentration of 500 μg/ml on the experimental day. Allopurinol (Sigma Aldrich, Schnelldorf, Germany) was suspended in a saline:Tween 20 solution (9:1) at 10 mg/ml and stored at 4 °C (one week shelf life). Uric acid (Sigma Aldrich, Schnelldorf, Germany) was prepared in modified Ringer's solution at a concentration of 1.6 mg/ml and was freshly made every day.

2.2. Animals

The male C57BL/6 JolaHsd mice that were used for the pharmacological experiments were obtained from Harlan (Horst, The Netherlands). Mice with genetically altered uric acid levels were obtained by targeting urate oxidase (UOx), the enzyme responsible for uric acid breakdown. Hemizygous *UOx* overexpressing (*UOx* OE) mice (C57BL/6 background) were obtained from Kenneth Rock, Department of Immunology, University of Massachusetts, Worcester, USA. The *UOx* transgene in these mice is driven by a strong constitutive (β-actin) promoter (Kono et al., 2010). Hemizygous *UOx* knock-out (*UOx* KO) mice were purchased from The Jackson Laboratory (B6;129S7/J background, Maine, USA) and crossbred. Offspring of these breedings were then genotyped for homozygosity and bred separately. The *UOx* KO mice have a constitutive disruption of the *UOx* gene, by insertion of a neomycin gene into exon 3. Allopurinol (200 mg/l) was provided in the drinking water of *UOx* KO breeding pairs and pups until 8 weeks of age to prevent lethality due to hyperuricemia (Wu et al., 1994). All mice were bred at the animal house of Ghent University Hospital and were housed at controlled temperature (21–22 °C) and relative humidity (40–60%) conditions. They had a fixed 12-hour light/dark cycle (lights-on period from 7 AM to 7 PM) and food and water available ad libitum. Treatment and care were in compliance with guidelines from the European Committee (decree 86/609/EEC). The study protocol was approved by the Animal Experimental Ethical Committee of Ghent University (ECD 09/61). All animals were 3–5 months old at the start of the experiments.

2.3. Induction of limbic seizures

Anesthetized mice were implanted with a custom-made polyimide-coated bipolar electrode (wire Ø 70 μm) with 0.5 mm tip separation, in which the deepest electrode contact was placed in the upper layer of the left dentate gyrus in the septal hippocampus (coordinates relative to bregma: anterioposterior: −1.8 mm; mediolateral: −1.0 mm; dorsoventral: −2.0 mm). An obturator-enclosed guide cannula was placed in the septotemporal transition zone of the hippocampus (coordinates relative to bregma: anterioposterior: −3.3 mm; mediolateral: −2.8 mm; dorsoventral: −2.3 mm). After a recovery period of one week the mice were connected to the EEG set-up and the guide cannula obturator was replaced with a microdialysis probe (CMA/7; 2 mm membrane length; theoretical cut-off 6 kDa; CMA, Kista, Sweden) which was continuously perfused with modified Ringer's solution at a flow rate of 1.25 μl/min. The next day, at 11 AM, a 500 μg/ml KA solution was infused for 3.2 min, after which the solution was switched back to modified Ringer's solution. With this protocol 2 μg KA was infused trough the microdialysis probe. Taking into consideration an estimated microdialysis probe recovery of 10%, we aimed to deliver 200 ng of KA, which is the dose frequently used for induction of status epilepticus by bolus injection (Bouilleret et al., 1999). This procedure results in a limbic status epilepticus with secondary generalized seizures and is not associated with any mortality (Fig. S1, Movies S1–5). A control group, in which mice were continuously infused with modified Ringer's solution, but not with KA, was included to control for potential effects of microdialysis on the uric acid level in the region of the probe.

2.4. Video-EEG analysis

Latency between the start of intrahippocampal KA infusion and the appearance of the first epileptic spike on the hippocampal EEG was determined as well as the total duration of epileptic activity. The latter parameter was defined as time between the first epileptic spike and the moment that spike activity dropped below a frequency of 1 Hz for more than 1 h (Grimonprez et al., 2015). Clinical seizures were counted in this period and scored based on a modified scale of Racine (Racine, 1972) (Movies S1–5): (stage 2) head nodding, (stage 3) unilateral forelimb clonus, (stage 4) bilateral forelimb clonus with rearing, (stage 5) rearing and falling, loss of postural control and (stage 6) running and bouncing. Clinical seizures with score 1 (extensive grooming, hyperactivity, eye closure, staring) were not included because they could not be discriminated from continuous abnormal background behavior. Clinical seizures with stage 2 were considered as partial seizures, and seizures with stages 3–6 were grouped as generalized seizures (Raedt et al., 2009).

2.5. Measurement of uric acid

Uric acid was quantified by HPLC using a Waters Alliance 2695 HPLC device (Waters, Zellik, Belgium) connected to a Waters 996 photodiode array detector according to an adapted protocol described by Fagugli et al. (2002). Data acquisition and processing were performed using Empower software (Waters). Analyses were performed on a reverse-phase XBridge C8 column (3.5 μm, 150 mm × 4.6 mm, Waters) with an Ultrasphere ODS guard column (5 μm, 5 mm × 4.6 mm, Beckman Instruments, Fullerton, CA). The chromatographic separation consisted of a linear gradient of methanol and ammonium formate buffer (50 mM, pH 3.0) from 0 to 100% methanol in 15 min at a flow rate of 1 ml/min. Forty-five microliters of dialysate was automatically injected. Uric acid was analyzed by UV detection at 300 nm. Standard solutions were used to perform calibration curves for uric acid quantification. For concentrations below the limit of detection (LOD; 0.112 μM) the LOD itself was used.

2.6. Protocol design

A schematic overview of the protocol design is shown in Fig. 1. Video-EEG monitoring and dialysate sampling started at 8.30 AM. Hippocampal dialysates were sampled in 50 minute periods. Each experiment started with the collection of two baseline samples in which only modified Ringer's solution was perfused. After KA infusion (or at the equivalent timepoint in the non-KA group) another six dialysates were collected. To test the electroclinical effects of lowering uric acid levels, animals were injected i.p. with either a 100 mg/kg suspension of allopurinol (ALLO) or its dissolving solution (9:1 saline:Tween 20 solution; SAL-T) 30 min before KA infusion. To test the electroclinical effects of uric acid increases, animals were infused in the left hippocampus with a 1.6 mg/ml uric acid solution for 200 min at a flow rate of 1.25 μl/min, starting 50 min before KA infusion. This was