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Research paper

Flupirtine effectively prevents development of acute neonatal seizures in an animal model of global hypoxia

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HIGHLIGHTS

- We examined efficacy of flupirtine to treat hypoxia-induced neonatal seizures.
- Flupirtine blocks development of behavioral seizures during hypoxia.
- Flupirtine treats clinical seizures and associated epileptiform activity.

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ABSTRACT

Current first-line drugs for the treatment of neonatal seizures have limited efficacy and are associated with side effects. Uncontrolled seizures may exacerbate brain injury and contribute to later-life neurological disability. Therefore, it is critical to develop a treatment for neonatal seizures that is effective and safe. In early-life, when the γ-aminobutyric acid (GABA) inhibitory system is not fully developed, potassium channels play an important role in controlling excitability. An earlier study demonstrated that flupirtine, a KCNQ potassium channel opener, is more efficacious than diazepam and phenobarbital for the treatment of chemoconvulsant-induced neonatal seizures. In newborns, seizures are most commonly associated with hypoxic-ischemic encephalopathy (HIE). Thus, in the present study, we examined the efficacy of flupirtine to treat neonatal seizures in an animal model of global hypoxia. Our results showed that flupirtine dose dependently blocks the occurrence of behavioral seizures in pups during hypoxia. Additionally, flupirtine inhibits the development of hypoxia-induced clinical seizures and associated epileptiform discharges, as well as purely electrographic (subclinical) seizures. These results suggest that flupirtine is an effective anti-seizure drug, and that further studies should be conducted to determine the time window within which it's administration can effectively treat neonatal seizures.

1. Introduction

Seizures are common in human neonates and are most frequently associated with hypoxia-ischemia [38]. Survivors of neonatal hypoxic-ischemic encephalopathy (HIE) often experience neurodevelopmental disabilities and seizures in later life [4,11,26]. Studies in both human neonates and animal models suggest that seizures themselves may independently contribute to brain injury and poor neurological outcome [5,18,29,31] (but also see Refs. [25,41]). Unfortunately, neonatal seizures are often resistant to treatment with approved antiepileptic drugs [15]. Throughout the world phenobarbital, an agonist of γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the adult brain, is the most commonly used drug for treating neonatal seizures [6,10]. The GABAergic system of the immature brain is underdeveloped compared to that of the mature brain, making it a sub-optimal target for the treatment of neonatal seizures [3,7,17,21]. Evidence from clinical and basic science research studies suggests that KCNQ potassium channels play a very important role in controlling excitation in early-life [33,35,36,39]. Flupirtine, a KCNQ channel opener [12,23,28,42], has been used clinically as an analgesic in Europe for over two decades with a good safety record. Our earlier study demonstrated that flupirtine is more efficacious than diazepam and phenobarbital for the treatment of chemoconvulsant-induced neonatal seizures [37]. In the current study, we evaluated the

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efficacy of flupirtine in the treatment of hypoxia-induced neonatal seizures in an animal model. The results suggest that flupirtine is effective in preventing development of both electroclinical and purely electrographic seizures during hypoxia.

2. Materials and methods

All animal procedures were performed in accordance with the NIH guidelines for the care and use of laboratory animals and according to the protocol approved by the Institutional Animal Care and use Committee (IACUC) of the University of Colorado Anschutz Medical Campus (UC-AMC). All efforts were made to reduce animal suffering and the number of animals used. Timed pregnant Sprague-Dawley rats were obtained from Charles River laboratories (Wilmington, MA). The pregnant rats were at the 14th day of gestation (E14) on arrival at the vivarium and delivered the pups at E22 or E23. The pups from both sexes were used for the study.

2.1. Hypoxia protocol

Ten day old (P10) rat pups were exposed to global hypoxia according to a published protocol [24]. The oxygen concentration of the chamber was maintained at 7% for 8 min, 6% for 4 min, 5% for 2 min and 4% for 1 min. The oxygen was balanced with nitrogen and the concentration of oxygen in the chamber was monitored using an oxygen sensor (Drager Pac 7000, Pittsburg, PA). The total hypoxia time varied slightly between the experiments, instead of exactly 15 min, as it took some time to equilibrate the chamber with the desired concentration of oxygen. During hypoxia, the temperature of the chamber was maintained at 36–37 °C, and the humidity at ~80%.

2.2. Flupirtine treatment protocol

In order to minimize the number of rats with electrodes for electroencephalography (EEG), as surgery for electrode implantation can cause short-term pain and distress to pups, a two-step approach was taken to examine the efficacy of flupirtine on behavioral and electrographic seizures. First, we identified a smallest drug dose that was most effective in preventing development of behavioral seizures during hypoxia (rats were not implanted to record EEG). For this, the rat pups were treated by intraperitoneal (i.p.) injection with either 25, 35, 45, and 50 mg/kg body weight flupirtine, or the vehicle (dimethyl sulfoxide and saline; 3:7 vol/vol) 15–30 mins before exposure to hypoxic environment, and monitored for signs of behavioral seizures during hypoxia. The flupirtine doses were selected based on our previous study where we observed that the 50 mg/kg flupirtine was effective in preventing chemoconvulsant seizures [37]. The smallest flupirtine dose that was most efficacious in preventing behavioral seizure was then chosen (35 mg/kg, i.p.) to determine if it also treats EEG correlate of behavioral (clinical) seizure, and purely electrographic (subclinical) seizures.

2.3. Electrode implantation for the video-EEG recording

At P9, the rat pups were implanted with the electrodes to record the electrical activity of the brain. One silver wire electrode (0.01 in. outer diameter; AM systems, Carlsborg, WA) was placed in each hemisphere of the brain over the parietal cortex. A silver electrode placed over the right and left side of the brain behind the lambdoid suture served as reference and ground electrodes respectively. The electrode assembly was held in place on the rat skull with tissue adhesive (3M Vetbond, St. Paul, MN) and dental acrylic cement. The entire implantation procedure was performed under isoflurane anesthesia (2–4% for induction and 1–1.5% for maintenance). After the surgery, the rats were returned to the dam and treated with analgesic (0.1 mg/kg buprenorphine hydrochloride) once every 12 h for 48 h.

2.4. Seizure monitoring and analysis

2.4.1. Behavioral seizures

The P10 rats were continuously monitored during hypoxia by an investigator (Yogendra Raol) and the occurrences of behavioral seizures were noted manually. The behavioral seizures (the rats were not implanted with electrodes to record EEG) consisted of head shakes, and clonic and tonic limb movements.

2.4.2. Electroclinical seizures

At P10, the rats were connected to an EEG monitoring unit (Stellate Harmonie system, Natus Medical, San Carlos, CA) to record EEG signals time-locked with digital video. The EEG signal was digitized at 1000 Hz and stored on a hard disk for offline analysis. Following a 20–30 min of baseline recording, the pups were given either vehicle or 35 mg/kg flupirtine by i.p. injection. After 15 min of video-EEG recording following the treatment, the pups were exposed to graded hypoxia. The pups were continuously monitored by video-EEG during the hypoxia. The video-EEG records were reviewed by a board certified clinical epileptologist (Andrew White) who was blinded to the treatment paradigm. Electroclinical seizures were defined by an EEG pattern that differed from background in either amplitude, frequency, or both, evolved over time, and contained spikes or sharp waves lasting for 10 s or more and were associated with a change in the rat's behavior. Electrographic seizures were defined as seizures observed in the EEG record that were not associated with a behavioral correlate on video.

2.5. Statistical analysis

GraphPad Prism statistical software (GraphPad Software Inc., San Diego, CA) was used for statistical analysis. Fisher's exact test and the Mann–Whitney test were used to determine the statistical significance of effects of flupirtine treatment on the frequency of rats developing seizures, and the amount of time spent in electroclinical seizures, respectively during hypoxia. P values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Flupirtine blocks development of behavioral seizures during hypoxia

To study the effect of flupirtine on hypoxia-induced seizures, the P10 rats were given various doses of the drug or vehicle 15–30 min before exposure to hypoxia. All of the pups that were treated with vehicle ($n = 12$; males (M) = 7, females (F) = 5) developed behavioral seizures during hypoxia (Fig. 1). The behavioral seizures consisted of head shakes, and clonic and tonic limb movements. Five out of eight pups ($n = 8$; 3M, 5F) treated with 25 mg/kg flupirtine did not exhibit any type of behavioral seizure; the remaining three rats developed head shakes during hypoxia (Fig. 1). None of the rats treated with 35($n = 5$; 3M, 2F), 45($n = 3$; 2M, 1F) or 50 ($n = 5$; 3M, 2F) mg/kg flupirtine developed behavioral seizures during exposure to the graded hypoxia (Fig. 1).

3.2. Flupirtine prevents development of electroclinical seizures during hypoxia

In neonates, there is often dissociation between clinical (behavioral) seizures and EEG phenomena, known as electroclinical uncoupling [19]. Because of this phenomenon, some drugs effectively treat the behavioral component of the neonatal seizure