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An investigation into the pharmacokinetics of 3-mercaptopropionic acid and development of a steady-state chemical seizure model using *in vivo* microdialysis and electrophysiological monitoring

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Summary
Objectives: The goal of the present study was to develop a chemical seizure model using the convulsant, 3-mercaptopropionic acid (3-MPA). A pharmacodynamics approach was taken, combining *in vivo* microdialysis sampling with electrophysiological methods to simultaneously monitor, in real-time, the 3-MPA concentration in the brain and the corresponding electrocorticographic (ECoG) activity.
Methods: The 3-MPA was administered in two doses (50 and 100 mg/kg) in order to study its pharmacokinetics. Microdialysis samples were collected from the striatum, hippocampus, and jugular vein every 5 min. The microdialysates were analyzed using high-performance liquid chromatography with electrochemical detection (HPLC-EC). The ECoG activity was monitored via screws placed onto the cortex. Noncompartmental pharmacokinetics analysis was performed to obtain the elimination constants (K_e), the maximum concentration (C_{max}), the time to achieve maximum concentration (T_{max}), and the area under the concentration—time curves (AUC_{inf}).
Results: The average brain K_e for the 50 and the 100 mg/kg doses were 0.060 and 0.018 min^{-1}, respectively. The brain AUC_{inf} for the 50 and 100 mg/kg doses were 353 and 2168 mg min^{-1} mL^{-1}, respectively. This led to a 67-fold increase in the observed number of seizures in the higher dose with the average seizure intensity double that of the smaller dose. These data led to

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the dosing scheme for the chemical seizure model of administering a 3-MPA loading dose of 60 mg/kg followed by a constant infusion of 50 mg/(kg min^{-1}).
Conclusions: This study describes, to our knowledge, the first successful attempt to combine *in vivo* microdialysis with electrophysiology to monitor in real-time, the concentration and effects of 3-MPA in the brain. This led to the development of a steady-state chemical seizure model.

Introduction

Epilepsy affects approximately 1% of the world population (Fisher and Coyle, 1991) of which an estimated 20% are resistant to current medications (Burnham et al., 2002). This explains the interest in seizure models, as they allow probing into mechanisms of seizure generation, which if fruitful, may translate into more efficacious therapies. Chemical seizure models provide information that is unique to them and easier to obtain, than that provided by other models such as kindling. The mechanisms of action and the seizures induced by 3-mercaptopropionic acid (3-MPA) (Sprince et al., 1969; Sprince et al., 1970; Loscher, 1973) have been well characterized (Skeritt and Johnston, 1983; Mares et al., 1993; Netopilova et al., 1997); 3-MPA decreases γ-aminobutyric acid (GABA) concentrations in the brain (de Lores Arnaiz et al., 1972; de Lores Arnaiz et al., 1973; Fan et al., 1981; Timmerman et al., 1992) by inhibiting glutamatic acid decarboxylase (GAD) (Lamar, 1970; Tunnicliff, 1990), that converts glutamate (Glu) to GABA. The imbalance between Glu and GABA, the main excitatory and inhibitory neurotransmitters in brain, respectively, manifests in this model as generalized seizures. Quantitative data about the changes in Glu and GABA, as a function of the concentration of 3-MPA in the brain, is lacking. If available, knowledge of the concentration of 3-MPA in the brain would serve as an important independent variable that can be used to investigate the type and degree of correlation between it and changes in the Glu/GABA ratio. Also, a correlation could be formed between the changes in Glu and GABA and the seizure frequency or rate and intensity.

The ability to monitor the concentration of 3-MPA in real-time, using *in vivo* microdialysis (Tossman and Ungerstedt, 1986; Robinson and Justice, 1991; Davies, 1999; de Lange et al., 2000; Ungerstedt et al., 2000; Weiss et al., 2000) would allow its regulation and achievement and maintenance of a steady-state concentration. This step will enable investigation of seizure frequency or rate and intensity, under conditions that either eliminate or allow precise tracking of fluctuations of the independent variable (convulsant concentration in the brain), thus allowing more accurate interpretation of results and better insight into seizure dynamics. Microdialysis sampling is a well-known technique that can be employed for monitoring *in vivo* pharmacokinetics. This technique involves implanting a probe containing a semi-permeable membrane into the brain or other organ of interest and collecting samples that will contain the analytes of interest. A solution that is isotonic to that of the cerebral spinal fluid (CSF) is constantly perfused through the implanted probe leading to no net loss of fluid. Microdialysis is an advantageous technique due to the semi-permeable membrane containing a particular molecular weight cut-off, which allows proteins and other large material to be excluded from the sample.

We describe to our knowledge, the first successful attempt to monitor *in vivo* and in real-time, the concentration and pharmacokinetics of 3-MPA in the brain and a dosing scheme for maintaining steady-state concentration in order to more accurately model the changes in neurotransmitters associated with epileptic seizures and how they may be correlated.

Methods

Animals

Male Wistar rats weighing 300—450 g (Charles River Laboratories, Wilmington, MA) were used. The animals were kept on 12 h light—dark cycles until the beginning of the experiment. Free access to food and water were allowed. The research described in this report was conducted in compliance with all applicable federal statutes and regulations related to animals and experiments involving animals and adheres to the principles stated in the Guide for the Care and Use of Laboratory Animals, NIH publication 86-23, 1996 edition.

Surgical procedure

Brain implantation of cortical electrodes, microdialysis guide cannula and probe

On the day of the experiment, rats were pre-anaesthetized with isoflurane. A subcutaneous injection of 67.5 mg/kg ketamine: 3.4 mg/kg xylazine: 0.67 mg/kg acepromazine was then administered for full anesthesia. Supplemental doses of 100 mg/mL ketamine were given at a rate of 0.2 mL/h to maintain the same plane of anesthesia. The anaesthetized rat was placed on a stereotaxic instrument (Harvard Appartus, Holliston, MA, USA) and then connected to a Homeothermic Blanket Control Unit (Harvard Apparatus, Holliston, MA, USA) where the body temperature was maintained at 37.0 ± 0.3 °C. A midline incision was made on the scalp and the skull was exposed. Four electrodes (1 mm o.d. stainless steel screws (Ace Hardware, Lawrence, KS, USA)) were placed over the cortex for recording of electrical activity. Two of the four electrodes were placed over the right hemisphere 4.2 mm anterior and 5.8 mm posterior and −1.4 mm lateral with respect to bregma; of the remainder electrodes, one was used as a ground electrode on the right hemisphere 5.8 mm posterior and +1.4 mm lateral with respect to bregma, and the other as reference (nasion).

Microdialysis intracerebral guide cannulas (CMA Microdialysis Inc., North Chemlsford, MA, USA) were implanted into the brain with the following coordinates: posterior 0.2 mm, lateral +3.2 mm, ventral 3.5 mm (striatum) and anterior 5.6 mm, lateral +4.8 mm, ventral 3.5 mm (hippocampus) with respect to the bregma (Paxinos and Watson, 1986). The guide cannulas were fixed to the skull surface with Duralay dental cement (Worth, IL, USA). A CMA/12 microdialysis probe with a 4 mm membrane (CMA Microdialysis Inc., North Chemlsford, MA, USA) was then placed through the guide cannula into both the striatum and hippocampus.