European Journal of Pharmaceutical Sciences 57 (2014) 25–33

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Correlation of 3-mercaptopropionic acid induced seizures and changes in striatal neurotransmitters monitored by microdialysis

Eric W. Crick [a], Ivan Osorio [b,c], Mark Frei [c], Andrew P. Mayer [a], Craig E. Lunte [a,*]

[a] R.N. Adams Institute for Bioanalytical Chemistry, Department of Chemistry, University of Kansas, 2030 Becker Drive, Lawrence, KS 66047, United States
[b] Comprehensive Epilepsy Center, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160, United States
[c] Flint Hills Scientific, LLC, 5040 Bob Billings Parkway, Suite A, Lawrence, KS 66049, United States

ARTICLE INFO

Article history:
Received 4 September 2013
Accepted 11 November 2013
Available online 22 January 2014

Keywords:
Status epilepticus steady-state chemical model
Microdialysis sampling
3-Mercaptopropionic acid
Catecholamines
Neurotransmitter amino acids

ABSTRACT

Objectives: The goal of this study was to use a status epilepticus steady-state chemical model in rats using the convulsant, 3-mercaptopropionic acid (3-MPA), and to compare the changes in striatal neurotransmission on a slow (5 min) and fast (60 s) timescale. *In vivo* microdialysis was combined with electrophysiological methods in order to provide a complete evaluation of the dynamics of the results obtained.
Objective: To compare the effects of a steady-state chemical model pof status epilepticus on striatal amino-acid and amine neurotransmitters contents, as measured via *in vivo* microdialysis combined with electrophysiological methods. Measurements were performed on samples collected every 60 s and every 5 min. "Fast" (60 s) and "slow" (5 min) sampling timescales were selected, to gain more insight into the dynamics of GABA synthesis inhibition and of its effects on other neurotransmitters and on cortical electrical activity.
Methods: 3-MPA was administered in the form of an intra-venous load (60 mg/kg) followed by a constant infusion (50 mg/kg/min) for min. Microdialysis samples were collected from the striatum at intervals of 5 min and 60 s and analyzed for biogenic amine and amino acid neurotransmitters. ECoG activity was monitored via screws placed over the cortex.
Results: In the 5 min samples, glutamate (Glu) increased and γ-aminobutyric acid (GABA) decreased monotonically while changes in dopamine (DA) concentration were bimodal. In the sixty second samples, Glu changes were bimodal, a feature that was not apparent with the 5 min samples. ECoG activity was indicative of status epilepticus.
Conclusions: This study describes the combination of *in vivo* microdialysis with electrophysiology to monitor the effect of 3-MPA on neurotransmission in the brain. This led to a better understanding of the chemical changes in the striatum due to the applied 3-MPA chemical model of status epilepticus.

1. Introduction

Epileptic seizures are known to result from imbalances within the neurotransmitter systems in the brain. Historically, epileptic seizures have been viewed as hyperexcitable events (Siegel et al., 1999; Nyitrai et al., 2006; Starr, 1996). This collection of research all point to the excitatory and inhibitory amino acid neurotransmitters, specifically glutamate (Glu) and γ-aminobutyric acid (GABA), and the biogenic amine neurotransmitters, in particular dopamine (DA) (Starr, 1996), as the main components of interest in gaining a more thorough neurochemical understanding of epilepsy. The interactions of Glu, GABA, DA, and all other important transmitters can be described as complex at best. Much work has been accomplished in order to understand the relationships between the amino acid and biogenic amine neurotransmitters in many different areas of the brain, including the striatum (Adams et al., 2002; Bert et al., 2002; Konradi, 1998; Shimizu et al., 1990; Takahata and Moghaddam, 2000), hippocampus (Clinckers et al., 2005), nucleus accumbens (Youngren et al., 1993), and ventral tegmental area (Chen and Rice, 2002; Karreman et al., 1996), but many of the observations are still unexplained.

A comprehensive understanding of the inter-relation between neurotransmitter systems and various epileptic seizure models is invaluable to the advancement of treatments for epilepsy and the development of new antiepileptic drugs (AEDs). The ability to better understand the neurophysiology of epilepsy and epileptic seizures would allow for better animal seizure models to be

* Corresponding author. Tel.: +1 785 864 3072; fax: +1 785 864 1916.
E-mail address: clunte@ku.edu (C.E. Lunte).

http://dx.doi.org/10.1016/j.ejps.2013.11.019
0928-0987/

developed which mimic more closely human epilepsy. Work recently completed in this laboratory produced a steady-state 3-MPA dosing model in which the concentration of 3-MPA was held at a steady concentration allowing for further evaluation of the neurotransmitter changes associated with the model (Crick et al., 2007). A good working knowledge of the neurotransmitter systems in relation to 3-MPA is non-existent. The ability to obtain data, regarding the changes in neurotransmission in different brain regions during the 3-MPA chemical seizure model, is critical in strengthening the developed model for its further use in the understanding of generalized seizures in the laboratory and clinical settings.

Fast neurochemical events have been monitored to transpire on the timescale of 0.5–5 ms (Siegel et al., 1999; Vyklicky et al., 1991). Many authors, beginning in the late 1970s with R.N. Adams (Adams, 1976; Wightman et al., 1978), have attempted to track these changes, primarily focusing on dopamine (DA), by electrochemical means (Budygin et al., 2000, 2001; Greco et al., 2006; Robinson et al., 2003). Other authors have also attempted to understand the role of certain ions, such as potassium and chloride, within neurological disorders (Gorji et al., 2006; Obrenovitch and Zilkha, 1995). While these typical carbon fiber (CF) electrodes perform very accurately at monitoring neurochemical changes, they are limited in functionality due to their inability to monitor multiple analytes simultaneously. This continues to be one area where microdialysis prevails. Microdialysis has recently been reported for monitoring neurochemical events at temporal resolutions ranging from 6 to 30 s (Bert et al., 2002, 1996; Parrot et al., 2003; Lada et al., 1997; Tucci et al., 1997).

We describe in this paper, the analysis of neurochemical changes using both High Performance Liquid Chromatography (LC) and Capillary Electrophoresis (CE) separation techniques and microdialysis. We also detail observations in the differences between the different microdialysis sampling frequencies during the 3-MPA induced seizure activity (model of convulsive status elipticus). Also discussed is the correlation between observed neurotransmitter activity and brain 3-MPA concentration. This is the first report, to our knowledge, that multiple changes in neurotransmitters have been correlated to 3-MPA induced seizure activity.

2. Methods

2.1. Chemicals/reagents

Monobasic sodium phosphate, disodium ethylenediamine tetraacetate (Na_2EDTA), 85% *o*-phosphoric acid, acetonitrile, methanol, hydrochloric acid (HCl), sodium hydroxide (NaOH), and 0.3 μm alumina powder were obtained from Fisher Scientific (Pittsburgh, PA). Ammonium acetate, 1-octanesulfonic acid [sodium salt] (SOS), sodium tetraborate decahydrate, boric acid, lithium tetraborate, lithium dodecyl sulfate (LDS), tetradecyltrimethylammonium bromide (TTAB), β-alanine (β-Ala), sodium cyanide, 4-hydroxybenzoic acid (4-HBA), L-glutamic acid (Glu), L-aspartic acid (Asp), L-arginine (Arg), γ-amino-*n*-butyric acid (GABA), DL-2-aminoadipic acid (AAP), sodium cyanide, 3, 4-dihydroxybenzylamine (DHBA), 3,4-dihydroxyphenethylamine hydrochloride (DA), L-arterenol (NE), homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) were obtained from Sigma–Aldrich (St. Louis, MO). Naphthalene-2,3-dicarboxaldehyde (NDA) was obtained from Invitrogen (Carlsbad, CA). All solutions were prepared in 18.2 MΩ distilled, deionized water (Labconco, Kansas City, MO) and filtered through 0.22 μm pore size membrane filters prior to use unless otherwise noted.

2.2. 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.

2.3. Surgical procedure

2.3.1. 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.

2.3.2. Femoral vein cannulation

A 25 mm length of MRE-033 tubing was inserted in the femoral vein and affixed properly by suturing. The skin incision was closed with tissue staples.

2.4. Experimental design

The animals were allowed to recover (under anesthesia at a rate of 0.2 mL of 100 mg/mL ketamine administered intramuscular (i.m.) every 1.5 h) for a period of 6 h. During this time, the environment surrounding the microdialysis probe is allowed to "recover" from the trauma suffered during the microdialysis probe implantation. It is well noted that during the time period following the implantation of a brain microdialysis probe that gliosis occurs (Plock and Kloft, 2005; Robinson and Justice, 1991). Also during this time period, the brain tissue function becomes disturbed due to the excess neurochemical release of numerous cellular storage compartments as well as increased glucose metabolism and decreased blood flow (Robinson and Justice, 1991). The time lapse between the implantation of the brain probe and the commencement of the experiment can last from 30 min to 24 h or longer depending