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Research article

Seizure liability assessments using the hippocampal tissue slice: Comparison of non-clinical species

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ABSTRACT

Introduction: Traditionally, rat hippocampal tissue slice models are used as an *in vitro* electrophysiology assay to assess seizurogenic potential in early drug development despite non-clinical species-specific differences noted during *in vivo* seizure studies.

Methods: Hippocampal tissue slices were acutely isolated from rats, minipigs, dogs and nonhuman primates (NHP). Population spikes (PS) were evoked through stimulation of the CA3 Schaffer collateral pathway and recorded using *in vitro* electrophysiological techniques *via* an extracellular electrode placed within the CA1 stratum pyramidale cell body layer.

Results: Hippocampal slices, across all species, displayed a concentration-dependent increase in PS area and number with the pro-convulsant pentylenetetrazol (PTZ; 0.1–10 mM). Beagle dogs exhibited higher sensitivities to PTZ-induced changes in PS area and number compared to both rats and NHPs which presented nuanced differences in their responsiveness to PTZ modulation. Minipigs were comparatively resistant to PTZ-induced changes in both PS area and number. Rat and NHP hippocampal tissues were further characterized with the pro-convulsant agents 4-aminopyradine (4-AP; 1–100 μM) and cefazolin (0.001–10 mM). Rats possessed higher sensitivities to 4-AP- and cefazolin-induced changes to both PS area and number whereas NHP displayed greater modulation in PS duration. The anti-convulsant agents, diazepam (10–500 μM) and lidocaine (1–500 μM), were also tested on either rat and/or NHP tissue with both drugs repressing PS activation at high concentrations.

Discussion: Hippocampal tissue slices, across all species, possessed distinct sensitivities to pro- and anti-convulsant agents which may benefit the design of non-clinical seizure liability studies and their associated data interpretation.

1. Introduction

Testing strategies and practices aimed at addressing adverse CNS effects represent an important aspect to the drug development process since they attempt to prevent serious adverse drug reactions from occurring within the clinical setting. These strategies commonly employ behavioral assays such as the Irwin assay (Irwin, 1968) and the functional observational battery (FOB) (Moser, Cheek, & MacPhail, 1995) as well as other more quantitative and objective measures such as electroencephalography (EEG) (Accardi et al., 2016; Authier et al., 2009, 2014; Durmuller, Guillaume, Lacroix, Porsolt, & Moser, 2007). Despite the successful implementation of these testing strategies within safety pharmacology, drug candidates continue to fail within clinical studies due to adverse CNS effects (*e.g.* drug abuse and dependence liability, cognitive impairment, seizures, suicidality) (Bass, Kinter, & Williams, 2004; Hamdam et al., 2013; Harrison, 2016) emphasizing the continued need to minimize drug attrition rates within pharmaceutical development (Bowes et al., 2012; Kola & Landis, 2004; Pangalos, Schechter, & Hurko, 2007; Waring et al., 2015). Drugs that reach the marketplace are also not without issue, a fact no better exemplified than by Minaprine, an anti-depressive agent, which, in 1996, was withdrawn from the market due to an increase in the clinical incidence of convulsions (Fung et al., 2001). Furthermore, seizures represent one of the most commonly encountered drug-induced preclinical CNS issues found in pre-clinical drug development (Authier et al., 2016). In 2016, 18% of the drugs approved by FDA were for neurology indications, the same proportion as was approved for oncology (Mullard, 2017). CNS drugs tend to be more often associated with CNS adverse events possibly due to higher brain penetration and high affinity for CNS constituents (Kesselheim, Wang, Franklin, & Darrow, 2015). Accordingly, there is growing support for more comprehensive CNS safety testing prior to clinical studies (Lindgren et al., 2008; Valentin & Hammond, 2008) especially when these assays can substitute work using animals.

In vitro brain slice electrophysiological methodologies are

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commonly employed to address adverse CNS effects within early drug discovery and safety testing (Accardi et al., 2016; Easter et al., 2009; Easter, Sharp, Valentin, & Pollard, 2007). Acute brain slice models, such as the hippocampal tissue slice model, represent a commonly employed *in vitro* electrophysiology preparation since it retains the *in vivo*-like stereotypic and temporal organization and structural integrity of neuronal microcircuits (Gibbs & Edwards, 1994). The hippocampus is recognized as a useful brain slice model due to the fact that it is easily extractable, can remain viable for several hours *ex vivo*, possesses a well-defined lamellar structure for ease of electrode positioning and orientation and represents one of the most well-studied brain regions in neuroscience (Accardi et al., 2016). The hippocampus is also strongly associated with partial (or focal) seizure induction (Schwartzkroin, 1994), an epileptic seizure in which the first clinical and EEG changes indicate initial activation of a system of neurons limited to part of one cerebral hemisphere (Chang, Leung, Ho, & Yung, 2017). Furthermore, the hippocampus plays a central role in temporal lobe epilepsy (Engel, 1996). Taken together, the hippocampus is well suited for use within seizure liability studies. Unsurprisingly, the hippocampal slice model has demonstrated strong concordance to *in vivo* exposure values of various drug candidates and known seizurogenic agents (*e.g.* antibiotics, antidepressants and antipsychotics) (Accardi et al., 2016; Dimpfel, Dalhoff, Hofmann, & Schluter, 1994; Easter et al., 2007, 2009; Fonck, Easter, Pietras, & Bialecki, 2015; Luchins, Oliver, & Wyatt, 1984; Markgraf et al., 2014; Oliver, Luchins, & Wyatt, 1982) with the rat hippocampal tissue slice assay displaying a predictability rate of 89% according to a validation dataset of 19 reference compounds (18 of which were known to induce seizures in man) (Easter et al., 2009). Nevertheless, in spite of this success, *in vitro* brain slice electrophysiology has yet to be consistently successful in predicting toxicities.

Traditionally, *in vitro* hippocampal electrophysiological assays have largely relied upon the rat hippocampal tissue which sometime possesses lower translational value to the human condition (Lynch & Schubert, 1980; Mead et al., 2016; Preuss, 2000). Accordingly, an assessment of hippocampal tissue isolated from several common animal models (*e.g.* rat, dog, minipig and nonhuman primate (NHP)) within a seizure liability assay would provide a better understanding of how differences amongst these species, often noted during *in vivo* seizure liability assessments and drug safety testing, may benefit the design of non-clinical seizure liability studies and their associated data interpretation. To this end, this investigation has begun to address these questions by assessing the performance of common animal model hippocampal tissue within an *in vitro* electrophysiology seizure liability assay using several clinically relevant pro- (pentylenetetrazol (PTZ), cefazolin, 4-aminopyridine (4-AP)) and anti- (diazepam, lidocaine) convulsant agents. In doing so, thisstudy sets out to highlight important differences to consider between non-clinical species as well as the complex nature of drug induced seizure-like activity; important issues within safety pharmacology.

2. Materials and methods

2.1. Statement on use and care of animals

During this investigation, care and use of animals were conducted in accordance with principles outlined in the current Guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care and the Guide for the Care and Use of Laboratory Animals published by the Institute of Laboratory Animal Resources. CiToxLAB's facility is AAALAC accredited and the procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) prior to conduct. All procedures were conducted as per Standard Operating Procedures (SOPs) in place.

2.2. Animals and housing conditions

All animals were housed under standard laboratory conditions with controlled temperature (21 ± 3 °C), humidity (30%–70%), 12 h light/dark cycle and 10–15 air changes per hour. Temperature and relative humidity were monitored continuously. Sprague-Dawley rats (2–4 months; Charles River Canada, St-Constant, Québec, Canada), cynomolgus monkeys (3–6 years; Guangxi Guidong Primate Development, China and Le Vallon Vieux Grand Port, Mauritius), Beagle dogs (6–14 months; Marshall Bioresources, North Rose, NY, USA) and Göttingen minipigs (6–9 months; Marshall Bioresources, North Rose, NY, USA) were used within this study. The animals were provided a standard certified commercial chow (Envigo Teklad Certified Global Rodent Diet #2018C, Hi-Fiber Primate Diet #7195C, 25% Lab Dog Diet #8727C and Miniswine Diet #7037C, respectively) and municipal tap water (which has been exposed to ultraviolet light and purified by reverse osmosis) *via* water bottles, automatic watering system and/or bowls, *ad libitum*.

2.3. Preparation of hippocampal tissue slices

Rats were anesthetized with isoflurane, *via* an induction chamber, and immediately decapitated using a rodent guillotine. Dogs, cynomolgus monkeys and minipigs were anesthetized with isoflurane followed by intubation and the whole brain was surgically removed. Upon extraction, the whole brain was rapidly submerged for a minimum of 1.5 min in modified oxygenated (95% O2, 5% CO2) ice-cold artificial cerebral spinal fluid (aCSF) solution, which contained (in mM): 124 NaCl, 3 KCl, 26 $NaHCO_3$, 2 $CaCl_2$, 1.3 $MgCl_2$, 10 glucose (pH 7.3 ± 0.1; 300–330 mOsmol/L). One or both of the hippocampi were subsequently isolated and sliced, using a vibrating tissue slicer (Leica VT100S, Leica Instruments), into 300–500 µm thick coronal or parasagittal slices maintained during the slicing procedures in oxygenated, ice-cold aCSF. On average, a total yield of approximately 6–12 slices were obtained per slicing procedure. The slices were transferred to oxygenated aCSF and held at room temperature (20 °C–23 °C) for at least 1 h before recordings were performed.

2.4. Electrophysiology

Slice experiments were performed at near physiological temperatures (33 °C–37 °C) on a Nikon Eclipse FN1 Upright Microscope (Nikon), equipped with differential interference contrast/infrared optics. Extracellular field recordings were performed on the hippocampal tissue slices using a bipolar stimulating electrode (CBBPE75; FHC, Bowdoin, ME, USA) placed in the CA2/3 stratum radiatum and the Schaffer collateral pathway with a borosilicate glass recording microelectrode (GC150F-10, OD 1.5 mm, ID 0.84 mm; Fisher Scientific; 0.5–4 MΩ, filled with 3 M NaCl) positioned within the CA1 cell body layer. The Schaffer collateral pathway was stimulated at 30 s intervals using constant current pulses (approximately 0.03 ms duration) of varying amplitudes such that evoked population spikes (PS) amplitudes were at least 0.3 mV (the mean PS amplitude was 1.45 ± 0.16 mV, n = 36). Recordings were made with a MultiClamp 700B amplifier (Molecular Devices) in current-clamp mode and Clampex 10.5 (Axon Instruments) was used to control the frequency of the voltage stimuli and to record the evoked response at the recording electrode. PS responses were filtered at 1 kHz and digitized at 25 kHz with a Digidata 1440A data acquisition board. The bath was continuously perfused at near physiological temperatures with aCSF at a rate of 1–3 mL/min. Slices were left to equilibrate for at least 20 min prior performing any *in vitro* electrophysiological recording.

A stimulation– and concentration-response relationship was determined for each slice. Each slice was stimulated, initially in the control aCSF solution, at 30 s intervals over the range 0–65 V (in 5 V increments) until a maximal PS amplitude was achieved. The stimulus

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