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INTRATHECAL APPLICATION OF THE MICROGLIAL INHIBITOR MINOCYCLINE ATTENUATES SYMPATHOEXCITATORY AND PROARRHYTHMOGENIC CHANGES IN RATS WITH CHRONIC TEMPORAL LOBE EPILEPSY

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Abstract—The incidence of sudden unexpected death in epilepsy (SUDEP) is highest in people with chronic and drug-resistant epilepsy. Chronic spontaneous recurrent seizures cause cardiorespiratory autonomic dysfunctions. Pituitary adenylate cyclase-activating polypeptide (PACAP) is neuroprotective, whereas microglia produce both pro- and anti-inflammatory effects in the CNS. During acute seizures in rats, PACAP and microglia produce sympathoprotective effect at the intermediolateral cell column (IML), whereas their action on the presympathetic rostral ventrolateral medulla (RVLM) neurons mediates proarrhythmogenic changes. We evaluated the effect of PACAP and microglia at the IML on sympathetic nerve activity (SNA), cardiovascular reflex responses, and electrocardiographic changes in the post-status epilepticus (SE) model of acquired epilepsy, and control rats. Chronic spontaneous seizures in rats produced tachycardia with profound proarrhythmogenic effects (prolongation of QT interval). Antagonism of microglia, but not PACAP, significantly reduced the SNA and the corrected QT interval in post-SE rats. PACAP and microglia antagonists did not change baroreflex and peripheral or central chemoreflex responses with varied effect on somatosympathetic responses in post-SE and control rats. We did not notice changes in microglial morphology or changes in a number of M2 phenotype in epileptic nor control rats in the vicinity of RVLM neurons. Our findings establish that microglial activation, and not PACAP, at the IML accounts for higher SNA and proarrhythmogenic changes during chronic epilepsy in rats. This is the first experimental evidence to support a neurotoxic effect of microglia during chronic epilepsy, in contrast to their neuroprotective action during acute seizures.

Key words: microglia, sympathoexcitation, temporal lobe epilepsy, rats, proarrhythmogenic, PACAP.

INTRODUCTION

Epilepsy is a chronic brain disorder characterized by spontaneous recurrent seizures and carries a risk of sudden death that is 15–20 times higher than in normal population (Ficker et al., 1998; Nilsson et al., 1999; Eastaugh et al., 2015). Epilepsy affects about 50 million people worldwide (WHO, 2005); seizures can range from brief, barely noticeable loss of attention to major convulsions that affect the entire neuraxis. Epilepsy is associated with changes in autonomic functions, such as sympathovagal imbalance, sympathetic reflex dysfunction, tachycardia with concomitant arrhythmia or bradycardia with associated apnea (Dütsch et al., 2006; Bateman et al., 2008; Ponnusamy et al., 2012; Massey et al., 2014; Powell et al., 2014b; Bhandare et al., 2015, 2016a). Seizure-associated autonomic cardiorespiratory changes are well-documented and are thought to play an important role in a mechanism of sudden unexpected death in epilepsy (SUDEP) (Nei et al., 2004; Dlouhy et al., 2015). Interictal autonomic changes are also seen in patients with chronic epilepsy (Ansakorpi et al., 2000; Berilgen et al., 2004; Müngen et al., 2010; Lotufo et al.,

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Abbreviations: AUC, area under curve; CD, cluster of differentiation; ECG, electrocardiogram; HR, heart rate; Iba1, iodinized calcium binding adaptor molecule-1; IML, intermediolateral cell column; Ir, immunoreactive; IT, intrathecal; KA, kainic acid; MAP, mean arterial pressure; O.D., outer diameter; PACAP, pituitary adenylate cyclase-activating polypeptide; $PaCO_2$, partial pressure of carbon dioxide; PBS, phosphate-buffered saline; Post-SE, post-status epilepticus; QT interval, a measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle; QTc, corrected QT interval; RVLM, rostral ventrolateral medulla; SE, status epilepticus; SNA, sympathetic nerve activity; SUDEP, sudden unexpected death in epilepsy; TH, tyrosine hydroxylase; TLE, temporal lobe epilepsy.

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2012). Nevertheless, the neuronal mechanisms causing autonomic cardiorespiratory dysfunction during chronic epilepsy are unknown.

Pituitary adenylate cyclase-activating polypeptide (PACAP), a 38 amino acid pleiotropic neuropeptide, produce neuroprotective effects (Shioda et al., 1998; Ohtaki et al., 2006; Bhandare et al., 2015) that are partly mediated through its action on microglia (Wada et al., 2013; Brifault et al., 2015). PACAP and microglia have a protective effect on sympathetic preganglionic neurons at the intermediolateral cell column (IML), where they ameliorate the sympathoexcitatory effect of acute seizures (Bhandare et al., 2015). During acute seizures, PACAP and microglia act on presympathetic rostral ventrolateral medulla (RVLM) neurons in the brainstem to promote proarrhythmogenic changes, but not sympathoexcitation (Bhandare et al., 2016a). In many cardiovascular autonomic nuclei PACAP is pressor and sympathoexcitatory (Farnham et al., 2008, 2011; Inglott et al., 2011) and changes baroreflex response in trout (Lancien et al., 2011) but not in rats (Farnham et al., 2012). PACAP expression is increased in central autonomic nuclei (paraventricular nucleus) after kainic acid (KA)-induced seizures in rats (Nomura et al., 2000). Secondly, seizures produce microglial activation, and neuroinflammation in patients and animal models (Beach et al., 1995; Shapiro et al., 2008; Eyo et al., 2014), which persist for many years during chronic epilepsy (Beach et al., 1995; Papageorgiou et al., 2015). Microglia can be pro- or anti-inflammatory in animal models of temporal lobe epilepsy (TLE) (Shapiro et al., 2008; Mirrione et al., 2010; Vinet et al., 2012; Devinsky et al., 2013). Although the pro- or anti-inflammatory state of activated microglia is a topic of debate, there is strong support for their dual role (Hanisch and Kettenmann, 2007). Short-term microglial activation is considered beneficial (Mirrione et al., 2010; Vinet et al., 2012; Szalay et al., 2016), whereas chronic microglial activation is deleterious, and produces a damaging response to injury (Qin et al., 2007; Loane et al., 2014; Olmos-Alonso et al., 2016). During KA-induced acute seizures, spinal microglia have a protective effect on sympathetic preganglionic neurons (Bhandare et al., 2015), however, their role in chronic epilepsy is not known.

Thus, the aims of this study were to identify the role of PACAP and microglia in the spinal cord, during chronic epilepsy, in the regulation of central autonomic cardiovascular activity. To achieve these aims we used a model of acquired epilepsy in rats that manifest spontaneous seizures and many features of acquired epilepsy in humans- the KA-induced post-status epilepticus (post-SE) model (Morimoto et al., 2004; Powell et al., 2008; Jupp et al., 2012). The effect of intrathecal (IT) infusion of the PACAP antagonist, PACAP(6–38), and the microglial antagonist, minocycline, on sympathetic activity, cardiovascular reflex responses, and the electrocardiogram (ECG) were analyzed in chronically epileptic and control rats. Microglial morphology and their phenotype in the vicinity of RVLM neurons were analyzed with immunohistochemistry in epileptic and control rats.

EXPERIMENTAL PROCEDURES

Animals

The animal usage and protocols were in accordance with the Australian code of practice for the care and use of animals for scientific purposes. The protocols were approved by the Animal Care, and Ethics Committee of Macquarie University, The University of Melbourne, and the Sydney Local Health District. The epilepsy surgery and procedures were performed under isoflurane anesthesia on 17–19-week-old adult non-epileptic control (n = 9), and post-SE (n = 15) male Wistar rats, whereas electrophysiology experiments were performed under urethane anesthesia.

KA-induced post-SE rat model

The post-SE model of acquired epilepsy was generated by i.p. injection of the glutamate receptor agonist, KA, to induce a period of continuous seizure activity (status epilepticus) in non-epileptic rats as described previously (Hellier et al., 1998; Powell et al., 2008, 2014b; Jupp et al., 2012; Vivash et al., 2014). Twelve-week-old Wistar rats were injected with repeated low doses of KA (5 mg/kg, i.p., followed by 2.5 mg/kg, i.p., injections once per hour) until SE behavior was observed. After 4 h of SE, all rats were given diazepam injection (5 mg/kg i.p.) to terminate the SE. Rats were then returned to their home cages in the animal house and maintained with normal animal house care and diet.

Implantation of EEG-ECG electrodes in post-SE and control rats

Seven weeks after KA-induced SE (n = 15) (or saline administered controls (n = 9)), two ECG electrodes and four EEG electrodes were implanted in each rat under isoflurane anesthesia (5% during induction, 2.5–1.5% for maintenance) in oxygen (2.0 L/min during induction, 0.5–1.0 L/min for maintenance) as detailed in supporting data by Powell et al. (2014b). Two small incisions were made to expose the thoracic muscle directly above the heart, and to expose the muscle overlying the xiphoid process of the sternum. The distal end of an ECG lead (13 cm; PlasticsOne, USA) was sutured to each muscle using polypropylene, 4-0 (Sharpoint, USA). The leads of both ECG electrodes were then tunnelled up through the left side of the neck subcutaneously, and the skin layer was sutured (polyglycolic, 4-0; LOOK™, USA). A single midline incision was then made on the scalp to expose the skull. Two ECG leads were located and tunneled through the neck to allow protrusion of the leads out of the incision site on the scalp. Each rat was then placed into a stereotaxic frame, and four extradural electrodes comprised of gold-plated sockets attached to stainless steel screws (O.D. 1 mm; Plastics One, USA) were implanted into the skull: one on each side approximately 2 mm from the midline, 3 mm anterior to the Bregma, one at approximately the center of the midline, and another 6 mm posterior to the Bregma, 4 mm right of the midline. The electrodes were fixed to the skull using

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