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Chronic antidepressant treatment accelerates kindling epileptogenesis in rats☆

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ABSTRACT

Objectives: Due to the high comorbidity of epilepsy and depression, antidepressant treatment is commonly indicated for patients with epilepsy. Studies in humans and animal models suggest that selective serotonin reuptake inhibitors (SSRIs) may reduce seizure frequency and severity, and these drugs are generally considered safe for use in epilepsy. No studies have investigated the effects of SSRIs on epileptogenesis, the neurobiological process underlying the development of the epileptic state.
Methods: The effect of continuous infusion of the SSRI, fluoxetine (10 mg/kg/day sc), versus vehicle control on amygdala kindling was examined in adult male Wistar rats. Seizure threshold and kindling rates were compared between SSRI-treated rats and controls. The study was then repeated examining the effect of a different SSRI, citalopram (10 mg/kg/day sc), versus vehicle control. Hippocampal mRNA expression of the serotonin transporter (SERT) and the 5-HT_{1A} receptor was examined in the brains of the rats post-mortem.
Results: Treatment with either fluoxetine or citalopram significantly accelerated kindling epileptogenesis, as evidenced by fewer stimulations to reach Class V seizures compared to their respective vehicle-treated group ($p < 0.01$ for both drugs). Seizure duration was also increased in fluoxetine-treated rats. No differences in seizure threshold were observed between treatments ($p > 0.05$). mRNA analysis did not reveal any molecular changes which were common to both treatments.
Conclusions: The rate of epileptogenesis in rats is enhanced by chronic treatment with SSRIs. This could potentially have implications regarding the effect of SSRIs on the development or progression of human epilepsy.

Introduction

Mood and anxiety disorders are common co-morbidities in patients with epilepsy, in particular those with drug-resistant epilepsy (Gaitatzis et al., 2004). Mesial temporal lobe epilepsy (MTLE), the most common form of drug-resistant epilepsy in adults, has a particularly strong association with depression (Kanner, 2006a). These mood disorders have a major adverse impact on the quality of life of patients, and are associated with poorer seizure control, increased drug resistance, and a higher likelihood of suicide (Hesdorffer et al., 2006). The presence of psychopathology in patients with epilepsy has been consistently found to be one of the most important determinants of quality of life (Boylan et al., 2004). Active management of depression and anxiety is therefore an essential part of comprehensive treatment of people with epilepsy.

The mainstay of the pharmacological treatment of mood disorders is antidepressant medication. There has been a commonly held concern amongst clinicians about the safety of the use of these medications in people with epilepsy, stemming from the propensity of 'first generation' antidepressants, particularly tricyclic antidepressants (TCAs), to induce seizures in a few patients (Salzberg and Vajda, 2001). However, a growing body of evidence suggests that this should be less of a concern for the newer, or 'second generation', antidepressants. These newer classes, notably the selective serotonin reuptake inhibitors (SSRIs), may even decrease seizure frequency (Alper et al., 2007; Bagdy et al., 2007; Kanner, 2009). These clinical reports largely agree with animal literature on the influence of SSRIs on experimentally-induced seizures (reviewed in Cardamone et al., 2013).

While previous clinical and experimental studies have examined effects of antidepressants on seizure frequency, to our knowledge, none have investigated the effects of these drugs on epileptogenesis, despite strong reasons to suspect such effects (Cardamone et al., 2013). Epileptogenesis refers to the neurobiological processes which transform normal brain networks into an epileptic state, with an increased

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propensity to generate seizures (Goldberg and Coulter, 2013). These processes continue even after the epileptic state is reached, and are likely compounded by the effects of the spontaneous recurrent seizures themselves.

There are several good reasons for considering effects of SSRIs on epileptogenesis, especially in MTLE. First, the brain structures where most epileptogenic changes occur in MTLE show considerable overlap with those involved with the neurobiology of mood disorders, notably the hippocampus and amygdala (Nestler et al., 2002). Secondly, serotonergic neurotransmission is a key aspect of the function of both these structures (Bagdy et al., 2007). Thirdly, there is increasing epidemiological evidence that depression and other psychopathologies pre-date, and are a risk factor for the emergence of epilepsy (Hesdorffer et al., 2012), and so the possibility should be considered that treatment with antidepressants of some depressed patients may influence the onset of epilepsy (Hesdorffer et al., 2000). Based on these and other considerations, an emerging theory proposes that the pathogenesis of depression and epilepsy share common mechanisms (Kanner, 2006b; Kanner, 2012), leading to the proposal that antidepressants may have antiepileptic efficacy (Kanner, 2012). The influence of antidepressants on epileptogenesis is thus a question of clear clinical importance: depression in epilepsy is often under-recognised and undertreated; authoritative bodies advocate screening and treatment to address this; however, a serious gap exists regarding knowledge of potential effects of antidepressants on epileptogenesis and disease progression.

With these issues in mind, this study was designed to investigate the effect of chronic SSRI treatment on the progression of epileptogenesis using the amygdala kindling rat model of temporal lobe epilepsy (Morimoto et al., 2004). We also investigated potential molecular mediators underlying any effects by studying key players in the mechanism of action of the antidepressants: the serotonin transporter (SERT) as the primary target of SSRIs, and 5-HT_{1A} receptors which may be relevant to depression in epilepsy (Hasler et al., 2007; Theodore et al., 2007). We tested the effect of two commonly prescribed SSRI antidepressants, fluoxetine and then citalopram, first-line medications for the treatment of depression in epilepsy.

Methods

Surgical implantation of osmotic pumps and stimulating/recording electrodes

Male Wistar rats aged 9–11 weeks underwent electrode implantation as previously described (Jones et al., 2009). Briefly, rats were anaesthetised with isofluorane (5% induction, 1.5–2.5% maintenance) and placed into a stereotactic frame. Holes were drilled into the skull to allow implantation of extradural recording electrodes, and a bipolar stimulating electrode (Plastics One, USA) into the left basolateral amygdala complex (AP: −3.0; ML: −5.25 relative to bregma; DV: −6.5 relative to the dura (Paxinos and Watson, 1998)). The assembly was held in place by dental acrylic (Vertex Dental, Netherlands). During the same surgery session, an osmotic minipump (Alzet, Cupertino, CA, USA) was implanted subcutaneously below the shoulder blades to facilitate continuous infusion of drug. We used 2ML4 pumps, which deliver ~2.5 μl/h for 28 days. Rats were allowed one week of recovery prior to the commencement of kindling. After kindling, the osmotic pumps were removed, and replaced with another pump which continued the drug treatment for a further 4 weeks. To facilitate our molecular biology experiments, the drug treatment was continued following the completion of kindling in order to dissociate permanent molecular effects of kindling and/or drug treatment from any transient effects caused by the final seizure. All procedures were approved by the University of Melbourne Animal Ethics Committee.

Drug treatments

We performed two sequential amygdala kindling experiments, each with one antidepressant drug and the vehicle comparator. In the first experiment, rats were randomly assigned to treatment with either fluoxetine hydrochloride (10 mg/kg/day, Aurobindo Pharma, India, n = 18) or vehicle (50% DMSO in sterile water, n = 16). The second experiment was designed as a replication experiment, but testing an alternate SSRI vs vehicle to determine whether any differences demonstrated between the treatment and control arms represented a drug class effect. For this, rats received either citalopram hydrobromide (10 mg/kg/day, Biotrend Chemicals, Switzerland, n = 25) or vehicle (50% DMSO in sterile water, n = 29) treatments. Doses were chosen based on previous literature in rats (Czachura and Rasmussen, 2000; Hesketh et al., 2005).

Seizure threshold test

Seizure threshold, defined as the minimum current required to elicit an electrographic discharge, was determined prior to kindling and again at the completion of kindling. EEG recording electrodes were attached to each of the extradural electrodes, which were connected to an amplifier and computer running LabChart software (ADInstruments, USA). Electrical stimulations (60 Hz, 1 s duration, 1 ms biphasic square wave pulse) were delivered to the amygdala via the bipolar electrode using an Accupulser Pulse Generator/Stimulator (A310, World Precision Instruments, USA). The initial current tested was 20 μA, and this was increased by 20 μA, 60 s seconds apart until an appropriate electrographic seizure (>6 s) was observed on the EEG (see Fig. 2A for example of electrographic event).

Amygdala kindling

The kindling procedure used in this study has previously been described (Jones et al., 2013), and adapted here with some modifications. Briefly, twice daily kindling sessions, separated by at least 4 h, were performed by electrically stimulating the bipolar electrode using the seizure threshold current unique to the particular animal. On the rare occasion when the stimulation did not evoke an electrographic event, the stimulating current was increased by 20 μA, and after a one minute delay, the animal was stimulated again. In all of these instances, this resulted in an electrographic seizure. Kindling was performed until all rats experienced 30 electrographic seizures. The Class (severity) of the behavioural seizures elicited by the stimulations were classified according to the Racine scale (Racine, 1972b), and the duration of the primary after-discharge was quantified from offline review of the EEG. Sham kindling consisted of identical handling to kindled animals but without electrical stimulation.

Post mortem procedures

Four weeks after the end of kindling, rats were given a 1 second continuous electrical stimulation at 2000 μA via the bipolar electrode to release ferrous ions at the electrode tip. This stimulation does not elicit a seizure, but facilitates the visualisation of electrode placement by interaction of the ferrous ions with the fixative solution. Rats were then given a lethal injection of phenobarbital (Lethabarb, 1 ml/kg ip) and cardiac blood taken for determination of plasma drug and metabolite concentrations. Rats were then transcardially perfused with 0.1 M phosphate-buffered saline followed by a fixative solution (4% paraformaldehyde/0.05% potassium ferrocyanide/0.05% potassium ferricyanide). Brains were removed, post-fixed in 4% PFA (24 h) and 30% sucrose (48 h) and frozen on dry ice. Serial coronal sections (20 μm) encompassing the hippocampus were cut on a cryostat, mounted on Superfrost Plus slides (Menzel-Glaser, Germany) and stored at −80 °C.