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Antidepressant drugs in convulsive seizures: Pre-clinical evaluation of duloxetine in mice

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ABSTRACT

Convulsive seizures (CS) are deleterious consequences of acute cerebral insults and prejudicial events in epilepsy, affecting more than 50 million people worldwide. Molecular mechanisms of depression and epilepsy include an imbalance between excitatory and inhibitory neurotransmission provoking oxidative stress (OS). OS is intimately linked to the origin and evolution of CS and is modulated by antidepressant and anticonvulsant drugs. Although newer antidepressants have exhibited a possible protective role in CS, studies analyzing serotonin and norepinephrine reuptake inhibitors merit to be further investigated. Thus, this study challenged the traditional model of pentylenetetrazol-induced CS, with only one administration of duloxetine. Male *Swiss* mice were treated with duloxetine (dose corresponding to the therapeutic range for human depression or greater, by allometric calculation; 10, 20 or 40 mg/kg), 30 min before pentylenetetrazol. Behavioral and electroencephalographic alterations were monitored. Lipid peroxidation, nitrites and catalase and superoxidase activities were measured in cortex. Behavioral and electroencephalographic results suggested a possible biphasic effect of duloxetine on CS, with anticonvulsant actions at therapeutic doses and a proconvulsant effect at higher doses. Duloxetine (20 mg/kg) also prevented lipid peroxidation and decreased catalase and superoxide dismutase activities in the cerebral cortex, with no influence on nitrites levels. These data demonstrated an anticonvulsant effect of duloxetine in CS for the first time. This extra anticonvulsant effect may allow the doses of anticonvulsants to be reduced, causing fewer side effects and possibly decreasing morbidity and mortality due to drug interactions in polytherapy.

1. Introduction

Seizures can originate as isolated responses to acute neurological insults or alterations in homeostasis in the brain due to acute disease, such as stroke and head trauma. Also, convulsive seizures are major events in epilepsy, a complex neurological disorder affecting roughly 50 million people worldwide (approximately 80% of them from developing countries (WHO, 2012), such as Brazil) and characterized by abnormal hypersynchronous neural activity. This abnormal activity manifests as chronic recurrence of unprovoked and spontaneous seizures, resulting in devastating effects on patients.

One of the most frequent psychiatric comorbidities in epilepsy is depression, which affects one out of every three patients with epilepsy (Kanner et al., 2012), but the molecular mechanisms are not completely understood. In the last decade, data have supported an association between the high comorbidity of the two disorders and common pathogenic mechanisms, such as decreased GABAergic neurotransmission and increased glutamatergic activity (Kanner, 2012). Interestingly, this imbalance between excitatory and inhibitory neurotransmission is intimately linked to the generation of

Abbreviations: CS, convulsive seizures; PTZ, pentylenetetrazol; SSRIs, selective serotonin reuptake inhibitors; SNRIs, serotonin and norepinephrine reuptake inhibitors; EEG, electroencephalogram; MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase; TCAs, tricyclic antidepressants; NERI, selective norepinephrine reuptake inhibitor; TTSS, total time spent in seizure.

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epileptic seizures (Staley, 2015) and is responsible for oxidative stress in both pathologies and comorbidity (Puttachary et al., 2015; Behr et al., 2012; Shin et al., 2011).

Oxidative stress is an imbalance between the generation of oxidant species and the activities of antioxidant defense systems, resulting in an overproduction of free radicals, such as reactive oxygen species and nitric oxide (Puttachary et al., 2015; Cardenas-Rodriguez et al., 2013). Patients with depression have decreased antioxidant enzyme activities and increased oxidative stress biomarkers (Behr et al., 2012). Oxidative stress is also intimately linked to epileptogenic processes, refractoriness to treatment in epileptic patients and worsening of the clinical status of patients with comorbidities (Puttachary et al., 2015; Cardenas-Rodriguez et al., 2013; Martinc et al., 2012; Rowles and Olsen, 2012). The deleterious consequences of oxidative stress on the central nervous system (CNS) have also been shown in several models of experimental epilepsy, such as amygdala kindling, pentilenetetrazol (PTZ) kindling, and acute PTZ-induced seizure models (Aguiar et al., 2013; Souza et al., 2013; Frantseva et al., 2000). Moreover, the therapeutic efficacy of anticonvulsant and antidepressant drugs is associated with the influence of these treatments on oxidative stress (Akpinar et al., 2014; Payandemehr et al., 2012; Martinc et al., 2012).

The association between antiepileptic drugs and antidepressants in patients is very common in clinical practice. Antidepressants are the third class of drugs most used by patients with epilepsy (Wilner et al., 2014). However, the use of antidepressants in epilepsy is controversial because of evidence supporting an increase in seizure severity, especially with older generations of drugs (e.g., tricyclic antidepressants (TCAs) and bupropion) (Jobe and Browning, 2005; Cardamone et al., 2013). Interestingly, some of the newer antidepressants, such as selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs), have been highlighted as potential anticonvulsant drugs (Jobe and Browning, 2005; Cardamone et al., 2013). A single dose of sertraline, for example, showed more potent anticonvulsant effects than similar doses of anticonvulsant drugs as carbamazepine, lamotrigine or phenytoin in a model with 4-aminopyridine (Sitges et al., 2016). The most studied among these drugs are the SSRIs citalopram and fluoxetine, especially in models with PTZ (Cardamone et al., 2013), which is currently considered a gold standard for screening possible anticonvulsant compounds (Souza et al., 2013; Yuen and Troconiz, 2015). However, results with both drugs have been contradictory, with studies reporting proconvulsant consequences.

On the other hand, the possible anticonvulsant effect of SNRIs has been scarcely studied, although previous studies with venlafaxine, the most studied SNRI, have demonstrated a potent action against convulsive seizures (Borowicz et al., 2011; Ahern et al., 2006). Still, high doses of venlafaxine (75 mg/kg or more) showed a proconvulsant effect (Santos et al., 2002). Also reboxetine, a selective norepinephrine reuptake inhibitor (NERI), has demonstrated effects in epilepsy (Ahern et al., 2006; Vermoesen et al., 2011, 2012; Popławska et al., 2015; Kumar et al., 2016).

In line with this idea, the preliminary results in our lab have indicated a possible role for the SNRI duloxetine in convulsive seizures (Coelho et al., 2014). No other work on duloxetine and its possible effects on seizures has been carried out except a recent report showing the efficacy of chronic treatment with duloxetine in a model of genetic absence seizure with depressive-like behavior (Citraro et al., 2015).

Therefore, the aim of this study was to challenge the traditional model of PTZ-induced convulsive seizures with only one administration of duloxetine using doses in the therapeutic range for human depression or above and to analyze a possible protective role of the drug against convulsive seizures and seizure-related oxidative stress.

2. Materials and methods

2.1. Animals and ethical aspects

Male *Swiss* mice (25–30 g) were housed under standard conditions (21 ± 2 °C; 12 h light/dark cycle) with food and water *ad libitum*. This study was conducted in accordance with the Ethical Principles of Animal Experimentation suggested by the NIH Guide for the Care and Use of Laboratory Animals and ARRIVE guidelines and it was approved by the Committee for Ethics in Experimental Research with Animals of the Federal University of Pará (license number BIO150-13). A total number of 98 animals, randomly grouped, were used in the present study: 58 for convulsive behavior and biochemical tests (n = 10 for PTZ-treated groups and n = 6 for groups without PTZ) and 40 for electrocorticographic recordings (n = 10 for all groups). Animals for electrocorticographic recordings were submitted to surgical procedures before any treatment (see below). No animal died naturally (without euthanasia) from all treatments carried out in the present study. All efforts were made to reduce the number of animals used and to minimize their suffering.

2.2. Surgical procedures

A set of animals was anesthetized with intraperitoneal Equithesin and placed in a rodent stereotaxic apparatus. Under stereotaxic guidance, two stainless steel screw electrodes were placed over the ipsilateral parietal cortex, along with a ground lead positioned over the nasal sinus. Bipolar nichrome wire teflon-insulated depth electrodes (100 μm) were implanted unilaterally into the ipsilateral hippocampus (coordinates relative to bregma: AP -4 mm, ML 3 mm, DV 3 mm). Electroencephalography was performed 3 days after surgery.

2.3. Treatments

All mice were treated intraperitoneally with duloxetine (10–40 mg/kg; Lili, USA) or saline. After 30 min, all animals were injected with PTZ (60 mg/kg i. p.; Sigma, Brazil) or saline (Souza-Monteiro et al., 2015) and electroencephalography and behavioral analysis of seizures carried out with continuous monitoring.

2.4. Electroencephalographic recordings

Before treatment, animals were transferred to a Plexiglas cage (25 × 25 × 40 cm) and habituated for 30 min before electroencephalography. Each animal was then connected to a 100× headstage pre-amplifier (model #8202-DSE3) in a low-torque swivel (Pinnacle Technology Inc, Lawrence, KS, USA) and the electroencephalogram (EEG) recorded using a PowerLab 16/30 data acquisition system (AD Instruments, Castle Hill, Australia). EEG signals were amplified, filtered (0.1–50.0 Hz, bandpass), digitized (sampling rate 1 kHz), and stored in a PC for off-line analysis. Routinely, a 10-min baseline recording was obtained to establish an adequate control period. After recording the baseline, animals were treated as described above. The animals were observed for the appearance of clonic and generalized tonic–clonic convulsive episodes for 20 min based on Ferraro et al. (1999), who described clonic convulsions as episodes characterized by typical partial clonic activity affecting the face, head, vibrissae, and forelimbs. Generalized convulsive episodes were considered to be generalized whole-body clonus involving all four limbs and tail, rearing, and wild running and jumping followed by sudden loss of upright posture and