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Research report

Subacute administration of fluoxetine prevents short-term brain hypometabolism and reduces brain damage markers induced by the lithium-pilocarpine model of epilepsy in rats

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ABSTRACT

The role of serotonin (5-hydroxytryptamine; 5-HT) in epileptogenesis still remains controversial. In this regard, it has been reported that serotonergic drugs can alter epileptogenesis in opposite ways. The main objective of this work was to investigate the effect of the selective 5-HT selective reuptake inhibitor (SSRI) fluoxetine administered subacutely (10 mg/kg/day × 7 days) on the eventual metabolic impairment induced by the lithium-pilocarpine model of epilepsy in rats. *In vivo* 2-deoxy-2-[18F]fluoro-D-glucose ([18F] FDG) positron emission tomography (PET) was performed to assess the brain glucose metabolic activity on days 3 and 30 after the insult. In addition, at the end of the experiment (day 33), several histochemical and neurochemical assessments were performed for checking the neuronal functioning and integrity.

Three days after the insult, a marked reduction of [18F] FDG uptake (about 30% according to the brain region) was found in all brain areas studied. When evaluated on day 30, although a hypometabolism tendency was observed, no statistically significant reduction was present in any region analyzed. In addition, lithium-pilocarpine administration was associated with medium-term hippocampal and cortical damage, since it induced neurodegeneration, glial activation and augmented caspase-9 expression. Regarding the effect of fluoxetine, subacute treatment with this SSRI did not significantly reduce the mortality rate observed after pilocarpine-induced seizures. However, fluoxetine did prevent not only the short-term metabolic impairment, but also the aforementioned signs of neuronal damage in surviving animals to lithium-pilocarpine protocol. Finally, fluoxetine increased the density of $GABA_A$ receptor both at the level of the dentate gyrus and CA1–CA2 regions in pilocarpine-treated animals. Overall, our data suggest a protective role for fluoxetine against pilocarpine-induced brain damage. Moreover, this action may be associated with an increase of $GABA_A$ receptor expression in hippocampus.

1. Introduction

Epilepsy is a chronic brain disorder characterized by the existence of recurrent episodic seizures. An abnormal increase in the neuronal excitability that frequently spreads to nearby areas is under the triggering of the different types of seizures (Reddy and Kuruba, 2013), resulting in alteration of the normal behavior. Epilepsy is one of the most common neurologic disorders, affecting almost 1–2% of the population. It is estimated that around 50 million of people worldwide suffer from epilepsy (Sander, 2003). Temporal lobe epilepsy (TLE) is the most common form of partial epilepsy in adults, and it usually presents a high ratio of pharmacoresistance.

Although classically, seizures have been attributed to the dysregulation of gamma-aminobutyric acid (GABA) and glutamate homeostasis (Alvestad. et al., 2011; Powell et al., 2014), serotonin (5-hydroxytryptamine; 5-HT) has been also involved in epileptogenesis. In this sense, the implication in epilepsy of this

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neurotransmitter was originally reported more than 50 years ago (Bonnycastle et al., 1957). Although the mechanisms ruling this function are still unclear, the presence of the different subtypes of 5-HT receptors controlling both excitatory and inhibitory synapses has been extensively described (Bagdy et al., 2007), playing a crucial role in the modulation of neuronal excitability. In line with this not clearly defined role, 5-HT and antidepressant drugs have been contradictorily associated with proconvulsant and anticonvulsant effects (Jobe and Browning, 2005).

During the last years, new animal models have been developed for the study and treatment of epilepsy (Martín and Pozo, 2006; Löscher, 2011). Among these models, the TLE model by administration of pilocarpine (or its variant consisting in previous administration of lithium) is one of the most employed animal models of epilepsy (Turski et al., 1983; Honchar et al., 1983; Turski et al., 1989; André et al., 2007). It has been reported to render most of the characteristics found in the human TLE, which in turn is one of the most common types of epilepsy in adulthood (Wieser, 2004). Indeed, animals show an acute and rapid status epilepticus (SE) after administration of pilocarpine. This stage is followed by a latency period, lasting approximately 30 days in which no seizures are detected. During this time, neuronal damage in hippocampus, cortex, amygdala and thalamus occurs (André et al., 2007). In a similar way to clinical epilepsy, this model has been reported to induce a sustained increase in cytosolic calcium, resulting in activation of the mitochondrial apoptosis pathway (Zhen et al., 2014). The aforementioned events along with an overproduction of reactive oxygen species (ROS) are believed to be involved in the neuronal death induced by pilocarpine insult (Liu et al., 2010). As a result, an aberrant neurogenesis and neural reorganization are developed (Müller et al., 2014; Shibley and Smith, 2002) which results in the appearance of spontaneous recurrent seizures (Moran et al., 2013). Finally, a severe but transient brain hypometabolism has been reported (Goffin et al., 2009; Guo et al., 2009).

Regarding the effects of fluoxetine, a well-known selective 5-HT reuptake inhibitor (SSRI), on this model of TLE, it has been reported to show anticonvulsant effects against the spontaneous seizure activity at long-term, when epilepsy had been established (Hernandez et al., 2002). Surprisingly, a single dose of fluoxetine administered shortly before the insult did not reduce, but increased the acute mortality rate associated with the pilocarpine-induced SE (Freitas et al., 2006).

With such a view, this study was aimed to elucidate the effects of a subacute treatment with fluoxetine, on the eventual metabolic and histochemical alterations during the silent period induced by the lithium-pilocarpine model of TLE in rats.

2. Materials and methods

2.1. Animals and ethical statement

Male adult Sprague-Dawley rats (Charles River Laboratories, Cerdanyola del Vallès, Spain) were used (weighing 365.1 ± 6.8 g immediately before the beginning of the study). Animals were housed in standard rat cages (2 animals/cage) on a ventilated rack (Tecniplast, Italy) under controlled temperature (20–24° C) and 12 h light/dark cycle. During the experiment, the animals had free access to standard rodent food (Safe, Augy, France) and tap water.

All procedures were carried out in accordance with animal welfare regulations of European Union (2010/63/UE) and Spain (RD53/2013). The study was approved by the Animal Research Ethical Committee of the Universidad Complutense de Madrid. All efforts were made in order to minimize the number of animals used and their suffering.

2.2. Epileptogenesis induction and drug treatment

The lithium-pilocarpine protocol was selected as experimental model of epileptogenesis (Kulkarni and George, 1995). The complete treatment protocol is schematized in Fig. 1. To investigate the subacute effects of fluoxetine on this experimental model of TLE, this drug (10 mg/kg, i.p.) was administered once daily for 7 days (from day −4 to day 2). The injection time was approximately at 10:00 am every day. Control animals received the same volume of distilled water as vehicle.

Twenty four hours before the induction of the epileptogenesis (day −1), lithium chloride was administered (3 mEq/kg (127 mg/kg), i.p.; Sigma–Aldrich, St. Louis, MO). The next day (day 0) and in order to reduce the cholinergic peripheral effects, the antimuscarinic agent methylscopolamine (2 mg/kg, i.p.; Sigma–Aldrich, St. Louis, MO) was administered 1 h after the corresponding fluoxetine (or vehicle) injection. Thirty min later, rats were injected with the convulsant agent pilocarpine (25 mg/kg, i.p.; Sigma–Aldrich, St. Louis, MO). The onset of SE was determined when an animal had stage 4 or 5 seizure according to Racine scale (Racine, 1972; Müller et al., 2014). Forty five min after the onset, pentobarbital was injected to rats (25 mg/kg, i.p.) for stopping seizure activity. If seizures did not discontinue, an additional half-dose of pentobarbital was administered 30 min later. The non-epileptogenic group received the same drug regimen; with the exception that saline was administered instead of pilocarpine. All drugs were administered at a volume of 1 ml/kg.

Two [^{18}F] FDG PET scans were performed on days 3 and 30 after the onset of the epileptogenesis. At the end of the experiment (day 33), the rats were sacrificed by decapitation, being their brains quickly removed. The samples were immediately frozen in dry-ice chilled isopentane and stored at −80 °C until further processing.

2.3. [^{18}F] FDG PET imaging

Two longitudinal [^{18}F] FDG PET studies were carried out to evaluate the evolution of the brain metabolic activity, on days 3 and 30. To this aim, a small-animal dedicated hybrid scanner was used (Albira ARS, Oncovision, Valencia, Spain). The acquisition and imaging processing protocols have been previously described (García-García et al., 2014). Briefly, fasted rats (at least 12 h) were injected i.v. into the tail vein with [^{18}F] FDG (approximately 18.5 MBq (500 μCi) in 0.2 ml of 0.9% NaCl; Instituto Tecnológico PET, Madrid, Spain). After an uptake period of 30 min, the animals were anesthetized by inhalation of a mixture of isoflurane/oxygen (5% for induction and 2% for maintenance) and placed on the bed of the scanner. PET acquisitions (20 min) were immediately followed by CT (computed tomography) scans. After the acquisitions, the tomographic images were reconstructed by applying an ordered subset expectation maximization algorithm for the PET and a filtered back projection for the CT images. For the metabolic activity quantification, the procedure used was as follows: first, the CT image of the skull from each animal was co-registered to a magnetic resonance image (MRI) rat brain template in which the regions of interest (ROIs) were previously drawn. Then, the spatial mathematic transformation was applied to its own fused PET image. This last step allows the correct matching between the PET image and the MRI template as described (Jupp and O'Brien, 2007). For these tasks, PMOD 3.0 software (PMOD Technologies Ltd., Zurich, Switzerland) was used. As index of regional metabolic activity, the standardized uptake value (SUV) was obtained. This quantifier was calculated applying the following expression: (ROI radioactivity concentration decay-corrected at the injection time (kBq/ml) × body weight (g)/injected dose (kBq)).