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Metyrapone prevents acute glucose hypermetabolism and short-term brain damage induced by intrahippocampal administration of 4-aminopyridine in rats

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ABSTRACT

Intracerebral administration of the potassium channel blocker 4-aminopyridine (4-AP) triggers neuronal depolarization and intense acute seizure activity followed by neuronal damage. We have recently shown that, in the lithium-pilocarpine rat model of status epilepticus (SE), a single administration of metyrapone, an inhibitor of the 11β-hydroxylase enzyme, had protective properties of preventive nature against signs of brain damage and neuroinflammation. Herein, our aim was to investigate to which extent, pretreatment with metyrapone (150 mg/kg, i.p.) was also able to prevent eventual changes in the acute brain metabolism and short-term neuronal damage induced by intrahippocampal injection of 4-AP (7 μg/5 μl). To this end, regional brain metabolism was assessed by 2-deoxy-2-[18F]fluoro-D-glucose ([18F]FDG) positron emission tomography (PET) during the ictal period. Three days later, markers of neuronal death and hippocampal integrity and apoptosis (Nissl staining, NeuN and active caspase-3 immunohistochemistry), neurodegeneration (Fluoro-Jade C labeling), astrogliosis (glial fibrillary acidic protein (GFAP) immunohistochemistry) and microglia-mediated neuroinflammation (*in vitro* [18F]GE180 autoradiography) were evaluated. 4-AP administration acutely triggered marked brain hypermetabolism within and around the site of injection as well as short-term signs of brain damage and inflammation. Most important, metyrapone pretreatment was able to reduce ictal hypermetabolism as well as all the markers of brain damage except microglia-mediated neuroinflammation. Overall, our study corroborates the neuroprotective effects of metyrapone against multiple signs of brain damage caused by seizures triggered by 4-AP. Ultimately, our data add up to the consistent protective effect of metyrapone pretreatment reported in other models of neurological disorders of different etiology.

1. Introduction

Epilepsy is a chronic neurological disorder characterized by enduring predisposition to generate seizures due to excessive electrical discharges in a group of brain cells and it can lead to severe brain damage and neurocognitive dysfunctions. The World Health Organization estimates that over 50 million people worldwide suffer from epilepsy (fact sheet no. 999 updated in February 2017 and available online at http://www.who.int/mediacentre/factsheets/fs999/en/). It is noteworthy that, even nowadays, around 30–40% of epileptic patients do not adequately respond to the current conventional pharmacological treatments. The lack of responsiveness in such a high percentage of patients is a relevant clinical problem due to the deleterious consequences that repeated exposure to seizures has on brain structure and function, particularly at the hippocampus.

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Substantial evidence supports that high plasma concentrations of glucocorticoids, such as those achieved under stress conditions, increases the vulnerability of hippocampal cells to damage (Sapolsky, 1985, 1996b). In fact, the hippocampus contains a high density of mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) (Joels and de Kloet, 1991). It seems that the deleterious central effects of glucocorticoids relay on multiple transcriptional and non-transcriptional GR- and MR-mediated mechanisms leading, among others, to enhanced glutamate-induced neurotoxicity and impaired neuronal glucose uptake and metabolism (Nasca et al., 2015; Sapolsky et al., 1985; Stein-Behrens et al., 1994a; Virgin et al., 1991). Hitherto, administration of metyrapone, a cytochrome P450 inhibitor that blocks the conversion of 11-deoxycortisol to the endogenous glucocorticoid corticosterone, has consistently shown hippocampal neuroprotective effects in several models of brain injury such as the status epilepticus (SE) induced by kainic acid (Stein and Sapolsky, 1988). Furthermore, metyrapone reduces the vulnerability to seizures and epileptogenesis in various rodent models of seizures of different etiology (Kaminski and Rogawski, 2011; Koe et al., 2014; Krugers et al., 1998). Likewise, we have recently reported that metyrapone pretreatment protects from the brain damage induced by SE in the rat lithium-pilocarpine model whereas metyrapone does not protect when administered at the time of SE, suggesting that the protective properties of metyrapone are of preventive nature (Garcia-Garcia et al., 2017). Metyrapone might also protect through mechanisms independent of its effects preventing the SE-evoked rise in plasma corticosteroid levels (Krugers et al., 1998). There are various other proposed glucocorticoid-independent mechanisms addressing the eventual neuroprotective effect of metyrapone (Drouet et al., 2012; Kaminski and Rogawski, 2011; Rotllant et al., 2002; Stein and Sapolsky, 1988). Among them, it is worth mentioning the role of metyrapone diverting the brain steroidogenesis pathway towards the synthesis of neurosteroids that, acting as positive allosteric modulators of $GABA_A$ receptors, promote inhibitory neurotransmission (Kaminski and Rogawski, 2011).

Thus, it seems worthy to extend the inquiry into the potential metyrapone-mediated neuroprotective effects in different animal models of seizures and SE (Martin and Pozo, 2006). In this context, 4-aminopyridine (4-AP; fampridine) is a non-selective blocker of voltage-dependent K^+ channels (K_v) commonly used in basic research as a convulsant agent. Acting as a depolarizing agent, increases action potential firing and neurotransmission leading to acute epileptiform activity (Hayes, 2004; Kovacs et al., 2003; Martin and Pozo, 2003; Yogeeswari et al., 2004). Curiously enough, and as long as a reasonable risk-benefit ratio regarding the likelihood of seizures is attained, is the depolarizing action what provides fampridine with the symptomatic effectiveness to treat multiple sclerosis and other neurological disorders (Hayes, 2004; Keune et al., 2015; Strupp et al., 2017).

One of the major factors contributing to 4-AP-induced seizures seems to be the release of the excitatory aminoacid glutamate and the stimulation of glutamate-mediated neurotransmission in multiple brain areas (including hippocampus, entorhinal cortex and striatum) (Medina-Ceja et al., 2000; Mihaly et al., 2001; Morales-Villagran and Tapia, 1996). This is further held up by the effect of glutamate antagonists administration protecting against epileptiform activity elicited by 4-AP (Doczi et al., 1999; Fragoso-Veloz and Tapia, 1992; Pena and Tapia, 1999, 2000). However, the degree of excitotoxicity and neuronal damage as consequence of glutamatergic hyperactivity-induced by 4-AP seems to be highly dependent on the way of administration of 4-AP. Thus, whereas peripheral administration of a low convulsant dose is associated to mild neuronal damage (Shiha et al., 2017; Slezia et al., 2004; Takacs et al., 2010), intrahippocampal injection encompasses intense brain damage, including loss of pyramidal neurons (Medina-Ceja et al., 2000; Pena and Tapia, 1999, 2000; Slezia et al., 2004; Takacs et al., 2010).

Altogether, intrahippocampal injection of 4-AP is widely accepted as a relevant model (Martin and Pozo, 2006) to study the pathological consequences of acute seizures and to test potential neuroprotective treatments. Clinical and basic neuroimaging studies in epilepsy reveal that alterations of brain metabolic activity are a common feature (Guo et al., 2009; Jupp et al., 2012; Meltzer et al., 2000; Shiha et al., 2015, 2017; Zilberter and Zilberter, 2017). Accordingly, our first aim was to study the consequences of a single intrahippocampal 4-AP injection on brain metabolic activity during the acute depolarizing phase by using *in vivo* 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) positron emission tomography (PET), a minimally invasive technique. Our second objective was to determine the short-term (3d after seizure) consequences of 4-AP injection on neuronal viability by evaluating different markers of neuronal integrity and brain damage. Finally, our third objective was to investigate whether and to which extent, a single administration of metyrapone, prior to the intrahippocampal injection of 4-AP, encompassed preventive neuroprotective properties as it does in other animal models of seizures.

2. Experimental procedures

2.1. Animals

Forty-four male adult Sprague-Dawley rats (Charles River Laboratories, Cerdanyola del Vallès, Spain) weighting 373.6 ± 5.8 g (85–90 d old) at the beginning of the experiment were used. Rats were housed in standard rat cages (2 rats/cage), on a ventilated rack (Tecniplast, Italy) under controlled temperature (22 ± 2 °C) and a 12 h light/dark cycle (0800 AM-0800 PM). Rats had *ad libitum* access to standard rodent chow (Safe, Augy, France) and tap water. To avoid an eventual circadian rhythm influence, all the procedures involving alive animals were carried out between 0800 AM and 0100 PM. The experimental procedures performed in the current study were approved by the Animal Research Ethical Committee of the Universidad Complutense de Madrid. They were in accordance with the European Union (2010/63/UE) and Spanish (RD53/2013) regulations regarding animal welfare and we made all efforts to minimize the number of animals used and their suffering.

2.2. Drug protocol and seizure induction

Anesthesia was induced by inhalation of 5% isofluorane and further maintained by using approximately 1.5% isofluorane in oxygen. For pain management, a local anesthetic cream (EMLA 25 mg/g + 25 mg/g lignocaine/prilocaine; AstraZeneca Farmacéutica Spain, S.A.) was applied at the incision site both, prior to and after surgery. The procedure took place in a stereotaxic frame and the rats were placed on a heating pad to prevent eventual hypothermia induced by isofluorane.

Seizures were induced by a single injection of 4-AP in the left dorsal hippocampus (7 μg/5 μl dissolved in 0.9% NaCl; ≈ 14.9 mM). This dose was selected based on *in vivo* studies showing that i.c.v. administration of 4-AP at doses ranging from 75 to 100 nmol in 5 μl (15–20 mM) produced convulsive behaviors characterized by bursts of forepaw clonic jerks and running fits (Folbergrova et al., 2016; Morales-Villagran and Tapia, 1996). In fact, in our study rats treated with 4-AP showed clear signs of convulsive crisis reaching the level of tonic-clonic seizures. Given the interference with the anesthesia administered before the PET scan we could not reliably evaluate the degree of severity achieved.

Non-insulted rats were injected with the same volume of saline