Neuroscience 328 (2016) 58–68

POST-TREATMENT WITH PROLACTIN PROTECTS HIPPOCAMPAL CA1 NEURONS OF THE OVARIECTOMIZED FEMALE RAT AGAINST KAINIC ACID-INDUCED NEURODEGENERATION

JULIO REYES-MENDOZA AND TERESA MORALES *

Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Querétaro, Qro. 76230, Mexico

Abstract—Kainic acid (KA) is a glutamate agonist widely used in studies of neurodegeneration due to its ability to induce excitotoxic damage in the rodent brain. Previously, we reported that pre-treatment with prolactin (PRL) prevents the neuron loss induced by KA administration in CA1, CA3 and CA4 of the hippocampus of the female rat. Here, we investigated if PRL has a neuroprotective effect in the dorsal hippocampus when it is administered after KA. For this, 100 ng of KA or 0.9% saline was administered intracerebroventricularly (ICV) to ovariectomized female rats. One hour later, they received subcutaneous PRL (103 μg/day for 7 days) or saline through an osmotic minipump. Also, to determine the hippocampal neurogenesis rate, the rats were administered bromodeoxyuridine along with the PRL treatment. Immunostaining for NeuN revealed that neuronal loss is lower in the CA1 of PRL-treated rats compared with the untreated group, but PRL did not confer any protection in the CA3 and CA4 subfields. Furthermore, PRL prevented the KA-induced cognitive deficit measured as a better performance in the novel object recognition test. The PRL treatment did not modify the neurogenesis rate. These data indicate that post-treatment with PRL confers differential neuroprotection against KA-induced neuronal loss in hippocampal subfield CA1, which correlates with a more mild cognitive deficit compared with the untreated control group.

Key words: hippocampus, excitotoxicity, prolactin, females, memory, neurogenesis.

INTRODUCTION

Excitotoxicity is a pathophysiological phenomenon involved in neuronal cell death after an insult to nervous tissue. This term, proposed by Olney in the 1960's, refers to neuronal death due to increased efflux of the excitatory amino acid neurotransmitter glutamate and consequent activation of its receptors (Olney et al., 1986; Sattle and Tymianski, 2001). The activated receptors provoke an exacerbated influx of calcium into the neuron and activate apoptotic and necrotic cell death pathways. This type of neuronal death is seen in several neuropathological conditions such as epilepsy, traumatic brain injury, hypoxic-ischemic damage, and other neurodegenerative diseases (Olney et al., 1986; Yi and Hazell, 2006; Dong et al., 2009).

A useful tool that mimics excitotoxic damage is the glutamate analog, kainic acid (KA) (Zheng et al., 2010), which due to its high affinity for glutamatergic kainate receptors is widely employed as a model of temporal-lobe epilepsy (Lévesque and Avoli, 2013). The presence of a high density of glutamate receptors makes the hippocampus particularly vulnerable to excitotoxic damage, and since this structure is involved in cognitive processes such as learning and memory, cognitive impairment reflects neuronal damage of this area (Mala et al., 2014). Several studies have shown that KA administration generates a selective neuronal loss in the hippocampus and consequent alterations in different hippocampus-dependent tasks like the Morris water maze (Stubley-Weatherly et al., 1996) and novel object recognition task (NOR) (Pearson et al., 2014).

Extensive research has been done in search of molecules, including hormones, that can reduce hippocampal neurodegeneration. The KA-lesioning model has been used to analyze the protective effects of hormones like progesterone (Ciriza et al., 2004), estrogens (Velísková et al., 2000), melatonin (Tan et al., 1998), and prolactin (PRL) (Tejadilla et al., 2010; Morales et al., 2014). In addition, neuroprotection is observed in the hippocampus of dams lesioned by KA during lactation (Vanoye-Carlo et al., 2008; Cabrera et al., 2009), a hyperprolactinemic reproductive phase in which cognitive improvement has been reported (Franssen et al., 2012).

Previous work from our laboratory has shown that pre-treatment with PRL diminishes hippocampal neurodegeneration caused by KA (Tejadilla et al., 2010; Morales et al., 2014) in ovariectomized (OVX) female

*Corresponding author. Address: Institute for Neurobiology, National Autonomous University of Mexico, Boulevard Juriquilla 3001, 76230 Queretaro, Qro., Mexico. Tel: +52-55-5623-4071; fax: +52-55-5623-4005.
E-mail address: marter@unam.mx (T. Morales).
URL: http://www.inb.unam.mx (T. Morales).

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BrdU, 5-bromo-2-deoxyuridine; DCX, doublecortin; ELISA, enzyme-linked immunosorbent assay; KA, kainic acid; KPBS, potassium phosphate buffer; NOR, novel object recognition; OVX, ovariectomized; PBS, phosphate-buffered saline; PRL, prolactin; PRL-R, PRL receptor; SOD, superoxide dismutase; SS, sterile saline.

http://dx.doi.org/10.1016/j.neuroscience.2016.04.030
0306-4522/

rats. Treatment with PRL or a phosphorylated mimic of the hormone reduced neuronal loss in the CA1, CA3 and CA4 areas of the hippocampus exposed to KA, and this neuroprotective effect of PRL correlated with decreases in the total score of behavioral seizure manifestations.

PRL is a hormone with numerous actions in nervous tissue (Freeman et al., 2000), where its effects are mediated by a wide array of mechanisms, including astrocyte alteration (DeVito et al., 1992, 1995a,b; Moderscheim et al., 2007; Arnold et al., 2014), antiapoptosis (Leff et al., 1996) and neurogenesis (Shingo et al., 2003; Torner et al., 2009). However, adult neurogenesis in the dentate gyrus of the hippocampus can also increase after a brain insult (Rola et al., 2006). Gray and Sundstrom (1998) showed that granule cell neurogenesis increases bilaterally 1 week after a single, unilateral intracerebroventricular injection of KA.

In many studies, potential protective treatments are given prior to the experimental lesion (Tejadilla et al., 2010; Frye and Walf, 2011). However, for an accidental injury or neurodegenerative diseases, the subject would need rescue treatment to minimize damage that may already be occurring. The present work aims to investigate: (a) if PRL could have a protective effect when administered after inducing the KA lesion, (b) if neurogenesis is involved in PRL actions, and (c) if the PRL treatment could reduce the cognitive deficit in a hippocampus-dependent task (Broadbent et al., 2009).

We found that post-injury treatment with PRL reduced the neuronal loss only in the CA1 hippocampal area and that such effect correlated with a better cognitive performance in a memory test. Neurogenesis was increased by the injury, but PRL treatment had no added effect.

EXPERIMENTAL PROCEDURES

Animals

Adult virgin female rats (180–200 g) were housed two per cage under controlled temperature and lighting conditions (12-h:12-h light:dark cycle, lights on at 06:00 h), with water and food *ad libitum*. All rats were OVX one month before the experimental procedures. Ovaries were surgically removed under ketamine/xylazine anesthesia (17.5/1.5 mg/200 g b.w., i.p.; Cheminova de México, Estado de México, México). Rats from each condition were randomly assigned to three groups: saline/saline (SS + SS), KA/saline (KA + SS) and KA/PRL (KA + PRL). The institutional Animal Care and Use Committee of the Institute of Neurobiology at the UNAM approved all experimental protocols. Animals were handled in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Official Mexican Guide of the Ministry of Agriculture (SAGARPA NOM-062-ZOO-1999).

Procedures

OVX rats were stereotaxically injected using a 10-μl Hamilton syringe with a screw to advance the plunger into the right ventricle (ICV, X: −1.9, Y: −1.3 and Z: −5.0, from Bregma) with either 100 ng of KA (Cabrera et al., 2009), or 1 μl of 0.9% sterile saline (SS) under deep anesthesia (ketamine–xylazine 17.5/1.5 mg/200 g b.w., i.p.). The administration rate was controlled by displacing one turn every 10 s for approximately 1 min of total time. After the injection, the ICV needle was left in place for 3 min to avoid reflux of the administered solution. One hour later, the animals received ovine prolactin (oPRL) (103.3 μg/day for 7 days; Sigma Aldrich, St. Louis, MO, USA) or saline through an osmotic minipump (Alzet, model 2001; Cupertino, CA, USA) implanted subcutaneously in the dorsal region of the subject's back. Treatment with this dose of PRL has been shown to be neuroprotective (Tejadilla et al., 2010). To detect neurogenesis, all subjects received 5-bromo-2-deoxyuridine (BrdU, 100 mg/kg b.w./day for 7 days, i.p.; Sigma Aldrich) along with the PRL treatment. Animals were sacrificed at two different time points: day 7 (7D group) and day 30 (30D group) after KA administration.

Behavioral tests

NOR test. Several studies have shown that NOR is a hippocampus-dependent task (Rampon et al., 2000). To evaluate if PRL treatment reduces the KA-induced cognitive deficit we applied this test to the 30D group (Fig. 1). We used the protocol described by de Lima et al. (2005) with some modifications. Briefly, the task took place in an acrylic black box with dimensions 50 × 50 × 30 cm. All animals received two habituation sessions, when they freely explored the arena for 8 min on days four and three before KA administration. On days one and two after the last habituation session, the animals were trained by allowing them 8 min to explore an area with two identical objects positioned in two adjacent corners at a distance of 10 cm from the walls, and ICV KA was administered on the following day. One day after KA injection, the animals were given the retention test, in which they explored the arena for 8 min in the presence of one familiar and one novel object. The same habituation, training, and retention protocol was performed again starting on day 25 after the lesion, with the retention test on day 30. The objects were counterbalanced among the subjects and trials. All procedures were registered with a video camera (Canon FS11A, Japan) for later analysis. The objects were: an orange glass bottle (5.5 cm diameter × 15 cm high) and a purple can of soda (6.5 cm diameter × 12 cm of high), and they were cleaned between trials with an alcohol–dextran solution (10%–10%) to avoid any olfactory cues. Exploration was defined as sniffing and touching the objects with the nose and/or forepaws. These parameters were quantified during the first 5 min of exploration because object discrimination is greatest during this period. The data are presented as exploratory preference expressed as percent: [Time attending object A/(time attending objects A + B)] × 100. Also, the difference between the exploratory preference for the novel object (Nov. Object) and the familiar object (Fam. Object) was calculated to determine whether there is a difference between the two training sessions. All behavior tests were recorded and then analyzed by blind observers.