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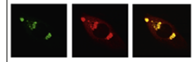

Research Report

Prolactin mediates neuroprotection against excitotoxicity in primary cell cultures of hippocampal neurons *via* its receptor

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ABSTRACT

Recently it has been reported that prolactin (PRL) exerts a neuroprotective effect against excitotoxicity in hippocampus in the rat *in vivo* models. However, the exact mechanism by which PRL mediates this effect is not completely understood. The aim of our study was to assess whether prolactin exerts neuroprotection against excitotoxicity in an *in vitro* model using primary cell cultures of hippocampal neurons, and to determine whether this effect is mediated *via* the prolactin receptor (PRLR). Primary cell cultures of rat hippocampal neurons were used in all experiments, gene expression was evaluated by RT-qPCR, and protein expression was assessed by Western blot analysis and immunocytochemistry. Cell viability was assessed by using the MTT method.

The results demonstrated that PRL treatment of neurons from primary cultures did not modify cell viability, but that it exerted a neuroprotective effect, with cells treated with PRL showing a significant increase of viability after glutamate (Glu) – induced excitotoxicity as compared with neurons treated with Glu alone. Cultured neurons expressed mRNA for both PRL and its receptor (PRLR), and both PRL and PRLR expression levels changed after the excitotoxic insult. Interestingly, the PRLR protein was detected as two main isoforms of 100 and 40 kDa as compared with that expressed in hypothalamic cells, which was present only as a 30 kDa variant. On the other hand, PRL was not detected in neuron cultures, either by western blot or by immunohistochemistry. Neuroprotection induced by PRL was significantly blocked by specific oligonucleotides against PRLR, thus suggesting that the PRL role is mediated by its receptor expressed in these neurons. The overall results indicated that PRL induces neuroprotection in neurons from primary cell cultures.

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1. Introduction

Prolactin (PRL) is a polypeptide hormone produced mainly in the anterior portion of pituitary gland, by the lactotrophs. While there is some evidence that it could also be synthetized in extra-pituitary sites, including some areas of the brain (Clapp et al., 1994; DeVito et al., 1991; Mejía et al., 1997; Torner et al., 2004), this still controversial (Grattan and Kokay, 2008; Kanyicska et al., 2000). PRL secretion is mainly inhibited by hypothalamus and it can be stimulated by suckling.

PRL displays several functions in the Central Nervous System (CNS), including the regulation of its own secretion through stimulation of TIDA neurons, stimulation of maternal behavior (Bridges and Mann, 1994; Bridges, 2014), suppression of fertility (Bouilly et al., 2012), and regulation of oxytocin neurons (Vega et al., 2010). Also, it has been described that PRL has several neuroprotective effects, either my stimulating neurogenesis and/or promoting survival, among which are: decreases neuroendocrine and behavioral responses to stress stimulus (Donner et al., 2007; Saltzman and Maestripieri, 2011), anxiolytic effects in male rats (Torner et al., 2001), protects hippocampal neurogenesis in the dentate gyrus of chronically stressed mice (Torner et al., 2009), induces neurogenesis in the sub ventricular zone in both *in vivo* and *in vitro* models (Gregg et al., 2007; Mohammad et al., 2002; Shingo et al., 2003), and enhances white matter repair and remyelination (Gregg et al., 2007).

The prolactin receptor (PRLR) is a class 1 receptor from the cytokine super family; it is composed of three domains: extracellular, transmembranal and intracellular (Bole-Feysot et al., 1998). Several PRLR isoforms have been described, generated by alternative splicing, proteolysis, intron retention, alternative sites for the end of transcription and partial exon suppression (Brooks, 2012; Ding and Wu, 2010). It is well documented that adult rodent brains express short and long PRLR isoforms (Tejadilla et al., 2010; Torner et al., 2009). In all isoforms, the extracellular domain is identical, but the downstream signal transduction activated by each isoform is different. The long form of the prolactin receptor is required for full activation of the prolactin receptor, including the well documents JAK/STAT5 pathway, while the short isoform can activate the MAP kinase pathway and induce mitogenic responses in some cell types.

Recent studies showed that the dorsal hippocampus of the rat is protected during lactation against excitotoxic damage induced by Kainic Acid (KA) (Cabrera et al., 2009, Vanoye-Carlo et al., 2008). The resistance to cell damage in hippocampal areas occurred either following peripheral or intracerebral administration of KA (Morales, 2011), suggesting that the brain adaptation during lactation modifies the sensitivity of this neural tissue to protect against excitotoxic insults. PRL is markedly elevated during lactation, and previous work has demonstrated that PRL exerts neuroprotective effects in the hippocampus in different experimental models (Franssen et al., 2012; Torner et al., 2009). Treatment with PRL, either through systemic or intracerebral administration, protects against the toxic effects of KA or reduces the epileptic like behavior induced by this glutamate agonist, and this effect is independent of ovarian hormones (Tejadilla et al., 2010; Vanoye-Carlo et al., 2008). Hence, PRL may mediate the neuroprotective effect seen during lactation.

In the present study, by using an *in vitro* model of primary culture of rat hippocampal cells we evaluated the neuroprotective role of PRL against glutamate, to test the hypothesis that the lactation-induced neuroprotective effect might be mediated by the hyperprolactinemia present at that time. We also examined the expression of PRLR in the cultures, to determine whether PRL actions might be mediated by its cognate receptor.

2. Results

2.1. *PRL did not modify cell neuronal viability in primary cell cultures*

Prolactin effect on neuronal cell viability was evaluated by MTT assay. The results indicated that PRL did not modify cell viability at any dose used (1–25 ng/mL). The prolactin effect was measured 72 h after hormonal treatment; the results are depicted in Fig. 1A.

2.2. *PRL protects hippocampal cells against excitotoxicity induced by glutamate*

PRL treatment had a preventive effect on glutamate-induced cell death compared with the glutamate-treated group. However, not all the PRL doses significantly prevented cell death induced by Glu, with the optimal dose being 10 ng/ml ($p<0.01$) (Fig. 1B).

2.3. *PRLR mRNA expression in primary cell cultures hippocampal cells*

PRLR expression pattern was assessed by RT-qPCR in all treatments groups. As depicted in Fig. 2, the basal expression of PRLR was determined in the primary neuronal cell cultures. Interestingly, PRL treatment increased PRLR expression. Furthermore, when cells were treated with PRL/GLU, the PRL-induced increase in PRLR mRNA was maintained. Interestingly, PRL mRNA was also detected in these cells, but at very low levels as compared to PRLR mRNA level (data not shown).

2.4. *Primary culture hippocampal cells express of prolactin receptor (PRLR)*

Primary cell cultures were labeled with NeuN to identify neural populations; more than 95% of cells were immunopositive for NeuN and these positive NeuN cell cultures were used in all subsequent experiments. Fig. 3A shows immunostaining with DAPI (A), NeuN (B), PRLR (C) and a merged image for these signals (D). PRLR was detected in almost 90% of the cells, predominantly in a cytoplasmic localization.

2.5. *PRLR isoforms expression on hippocampal cells*

Western blot analysis of hippocampal cells revealed the expression of two isoforms of PRLR with molecular weights of 100 and 40 kDa, potentially corresponding to long and short isoforms of PRLR, respectively (see Fig. 4). Mammary gland was used as positive control for PRLR, which express variants of PRLR. A hypothalamic derived cell line from mouse (mHypoE-N42 cell line, from Cell Solutions, Canada) presented a shorter

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