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INHIBITION OF PROLACTIN WITH BROMOCRIPTINE FOR 28 DAYS INCREASES BLOOD–BRAIN BARRIER PERMEABILITY IN THE RAT

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Abstract—The blood–brain barrier (BBB) is necessary for the proper function of the brain. Its maintenance is regulated by endogenous factors. Recent evidences suggest prolactin (PRL) regulates the BBB properties *in vitro*, nevertheless no evidence of these effects have been reported *in vivo*. The aim of this study was to evaluate the role of PRL in the maintenance of the BBB in the rat. Male Wistar rats were treated with Bromocriptine (Bromo) to inhibit PRL production for 28 days in the absence or presence of lipopolysaccharide (LPS). BBB permeability was evaluated through the Evans Blue dye and fluorescein-dextran extravasation as well as through edema formation. The expression of claudin-5, occludin, glial fibrillary acidic protein (GFAP) and the PRL receptor (PRLR) was evaluated through western blot. Bromo reduced the physiological levels of PRL at 28 days. At the same time, Bromo increased BBB permeability and edema formation associated with a decrement in claudin-5 and occludin and potentiated the increase in BBB permeability induced by LPS. However, no neuroinflammation was detected, since the expression of GFAP was unchanged, as well as the expression of the PRLR. These data provide the first evidence that inhibition of PRL with Bromo affects the maintenance of the BBB through modulating the expression of tight junction proteins *in vivo*.

Key words: prolactin, blood–brain barrier, lipopolysaccharide, tight junctions, permeability.

INTRODUCTION

The blood–brain barrier (BBB) can be defined as the frontier that separates the brain tissue from the circulating substances in the vascular system (Bernacki et al., 2008). The BBB is mainly formed by endothelial cells, which have specific properties that set them apart from other endothelial cells in the body, including a low pinocytic activity, lack of fenestrations, high mitochondrial content and high density of tight junctions (TJs) (Hawkins and Davis, 2005; Weksler et al., 2005; Cardoso et al., 2010). The brain endothelium is responsible for the regulation of the selective transport of substances from blood to brain and vice versa (Persidsky et al., 2006) and to maintain the high electrical resistance and low permeability due to the presence of TJs. The TJs are formed by a collection of transmembrane proteins, being claudin-5 and occludin the most important in the BBB. These proteins homotypically interact with extracellular domains of claudins and occludins from neighboring cells and with scaffold proteins like zonula occludens-1 and -2 with their intracellular domains. At the same time, these scaffold proteins interact with the actin cytoskeleton resulting in the tight union between adjacent endothelial cells in the BBB (Ebnet, 2008; Forster, 2008; Liu et al., 2012), which in conjunction maintain the brain homeostasis and proper function of the central nervous system under physiological conditions. Several endogenous molecules regulate the BBB properties, like the vascular endothelial growth factor (VEGF) or Wnt 7a and 7b among others. These molecules are key factors in the development and acquisition of the BBB phenotype (Liebner et al., 2008; Tam and Watts, 2010). In addition, it has been reported that glucocorticoids (Sadowska et al., 2010; Salvador et al., 2014) and hormones like estrogens (Kang et al., 2006), growth hormone (GH) (Muresanu et al., 2010) and prolactin (PRL) (Rosas-Hernandez et al., 2013a,b) can modulate different features of the BBB both *in vitro* (Cucullo et al., 2004; Rosas-Hernandez et al., 2013a,b) and *in vivo* (Kang et al., 2006; Muresanu et al., 2010). These last two hormones are members of the PRL family. PRL is a 199-amino acid, 23-kDa polypeptide hormone produced mainly by the lactotrophs in the anterior pituitary. Its production is regulated by dopamine through activation of the D2 receptors, in a short-loop feedback mechanism; therefore, its synthesis can be inhibited with D2 receptor agonists like Bromocriptine (Bromo) (Freeman et al., 2000). PRL is usually related with milk production (Freeman et al., 2000); however, more than 300 functions have been attributed to this hormone (Bole-Feysot et al.,

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Abbreviations: AF, amniotic fluid; BBB, blood–brain barrier; bBMVECs, bovine brain microvessel endothelial cells; Bromo, bromocriptine; DMF, N,N-dimethylformamide; EB, Evans Blue; FITC, fluorescein isothiocyanate; GFAP, glial fibrillary acidic protein; GH, growth hormone; HRP, horseradish peroxidase; LPS, lipopolysaccharide; Meth, methamphetamine; PRL, prolactin; PRLR, PRL receptor; TEER, trans endothelial electrical resistance; TJs, tight junctions; VEGF, vascular endothelial growth factor.

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1998). Among these functions, PRL regulates the permeability in different cell types *in vitro*, including the kidney epithelium (Peixoto and Collares-Buzato, 2006) and the mammary epithelium, through modulating the expression of TJ proteins (Stelwagen et al., 1999; Ueda et al., 2011). *In vivo,* this hormone modulates the permeability to different substances in the mammary gland (Linzell et al., 1975; Flint and Gardner, 1994; Nguyen et al., 2001). However, in the BBB, the effects of the members of the PRL family have been poorly studied. We first reported in primary cultures of bovine brain microvessel endothelial cells (bBMVECs) that high concentrations of PRL increase the trans endothelial electrical resistance (TEER) associated with a decrease in BBB permeability and with an increase in the expression of the TJ proteins claudin-5 and occludin, without changes in the expression of the PRL receptor (PRLR) (Rosas-Hernandez et al., 2013a). Moreover, using the same *in vitro* model of BBB, we demonstrated that PRL protects against the methamphetamine (Meth)-induced increase on permeability through the up-regulation of the expression of claudin-5 and occludin (Rosas-Hernandez et al., 2013b). Despite all these previous evidences, there are no studies about the effect of PRL on the formation and maintenance of the BBB *in vivo*. The aim of this study was to evaluate the effects of PRL on the maintenance of the BBB and the mechanisms involved in this process, by inhibiting its production on male Wistar rats using the D2 receptor agonist, Bromo.

EXPERIMENTAL PROCEDURES

Materials

2-Bromo-α-ergocryptine methansulfonate salt, lipopolysaccharides (LPSs) from *Escherichia coli*, Evans Blue (EB) dye, fluorescein isothiocyanate (FITC)-dextran MW 20,000 Da, antibody against the glial fibrillary acidic protein (GFAP) and Horseradish peroxidase (HRP)-secondary antibodies were purchased from Sigma chemical company (St. Louis, MO, USA), rat PRL ELISA kit was purchased from ALPCO (Salem, NH, USA), saline solution was purchased from Pisa laboratories (Guadalajara, Mexico), N,N-dimethylformamide (DMF) was purchased from Fermont laboratories (Monterrey, Mexico), antibodies against claudin-5 and the PRLR were purchased from Abcam (Cambridge, MA, USA), while the antibody against occludin was purchased from Invitrogen (Carlsband, CA, USA). All the remaining reagents were purchased from Sigma chemical company (St. Louis, MO, USA).

Animals

Male Wistar rats (250–300 g) were used in all the experiments. Rats were housed in clear plastic containers (2 per cage), under a 12-h dark/light cycle with *ad libitum* access to water and food. All the experiments were performed in accordance with the National Institutes of Health Guide for the Use and Care of Laboratory Animals guidelines and approved by the Animal Care and Use Committee from the Faculty of Chemistry of the Universidad Autonoma de San Luis Potosi, Mexico.

Treatments

To analyze the effect of PRL inhibition, male Wistar rats were randomly divided in 4 groups ($n = 8$ animals per group). Group 1 received i.p. injections of saline once a day for 28 days, group 2 received i.p. injections of saline once a day for 28 days and on day 29 received a single dose of LPS (1 mg/kg i.p.), group 3 received Bromo (1 mg/kg i.p.) once a day for 28 days and group 4 received Bromo (1 mg/kg i.p.) once a day for 28 days and on day 29 received a single dose of LPS (1 mg/kg i.p.).

PRL quantification

The systemic PRL quantification was done 2 h after the administration of LPS or 24 h after the last administration of Bromo or saline. The animals were anesthetized with sodium pentobarbital (40 mg/kg i.p.) to minimize animal suffering. 500 μL of blood was extracted from the jugular vein and was centrifuged (5590*g* for 10 min at 4 °C), plasma was separated and stored at −80 °C until analysis. PRL quantification was determined with a commercial kit following the manufacturer instructions. Briefly, 25 μL of plasma samples was pipetted into a 96-well plate coated with anti-rat PRL antibody, incubated for 2 h at room temperature, washed 4 times and then incubated for 1 h with enzyme-labeled anti-rat PRL antibody. After 4 washings of the plate, 3,3′,5,5′-tetramethylbenzidine substrate solution was added and incubated for 30 min in the dark at room temperature. Finally, 50 μL of 2 M hydrochloric acid was added and the absorbance was read at 450 nm with a reference wavelength of 650 nm. Values were interpolated in a rat PRL calibration curve and were expressed as ng of PRL/mL.

EB extravasation

To analyze BBB disruption the EB technique was employed; this dye binds to albumin that only crosses the BBB when there is an increase in its permeability (Sharma and Ali, 2006). After the animals were anesthetized and the blood samples were collected, a solution of EB dye (45 mg/kg) was injected into the jugular vein and allowed to circulate in the bloodstream for 2 h. After that, the animals were euthanized and transcardially perfused with 150 mL of PBS at pH 3.0. The brains were removed and separated into right and left hemisphere to minimize the number of animals used, since there was no difference in appearance between the two hemispheres (Northrop and Yamamoto, 2012). One hemisphere was used to determine the EB extravasation and the other one to determine the protein expression, in order to evaluate both effects in the same brain. The right hemisphere was immersed in 1 mL DMF and incubated for 18 h at 80 °C and then centrifuged (9740*g*, 1 h, 4 °C). The supernatant was collected and the absorbance was measured at 620 nm. Values were interpolated in a