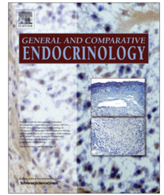
General and Comparative Endocrinology 189 (2013) 105–110

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen



Metoclopramide-induced hyperprolactinemia effects on the pituitary and uterine prolactin receptor expression

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ARTICLE INFO

Article history:
Received 19 February 2013
Accepted 30 April 2013
Available online 14 May 2013

Keywords:
Hyperprolactinemia
Progesterone
Estrogen
Prolactin receptor
Mice

ABSTRACT

In this work we have evaluated the gene expression profile of prolactin and prolactin receptor in the pituitary and the uterus of female mice with metoclopramide-induced hyperprolactinemia treated with estrogen and/or progesterone. For this purpose, 49 Swiss female mice were allocated to seven groups. Interventions: 50-day treatment with metoclopramide, progesterone and estrogen. Our results showed that in the pituitary, metoclopramide-induced hyperprolactinemia increased prolactin expression. In the castrated animals, progesterone, with or without estrogen, produced an increase in prolactin. Pituitary prolactin receptor and the estrogen and progesterone treatment were responsible for the rise in PRLR-S2. In the uterus, no differences in prolactin expression were found between the different study groups. PRLR-S1 had its expression reduced in all castrated animals as against the castrated group treated with vehicle. In the noncastrated animals, PRLR-S2 rose in the metoclopramide-treated group, and, in the castrated animals, its expression diminished in all groups in relation to the vehicle-treated castrated controls. An increase in PRLR-S3 was found in the oophorectomized animals treated with a combination of estrogen and progesterone. PRLR-L rose in the oophorectomized animals treated with progesterone in isolation or in association with estrogen. These findings suggest that metoclopramide associated to progesterone or estrogen may determine an increase in pituitary prolactin and PRLR-S2 expression. The estrogen-progesterone may enhance the expression of PRLR-S3 and PRLR-L isoform of prolactin receptor.

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1. Introduction

Hyperprolactinemia is the most common endocrine disorder productive of hypersecretion on the hypothalamic-pituitary axis. It occurs predominantly in young women (20–30%) and can lead to several abnormalities, including infertility (Rossi, 2009). Hyperprolactinemia is characterized by high levels of prolactin (PRL) – a simple peptide of approximately 200 amino acids – in the blood; its biological effects are mediated by its interaction with its receptor (PRLR) Rossi et al., 2002.

The PRLR belongs to the superfamily of type 1 cytokine receptors (Bole-Feysot et al., 1998), and isoforms are generated by the splicing of RNA from only one gene or through post-traductional modifications. In mice a long isoform (PRLR-L) and three short ones (PRLR-S1, PRLR-S2, and PRLR-S3) are known. Expression of these isoforms may vary according to the estrous cycle, and it is also associated with high PRL levels in the circulation (Binart et al., 2010; Casanueva et al., 2006).

In mice it is possible to mimic hyperprolactinemia symptoms by administering metoclopramide. This drug acts on the specific dopamine D-2 receptor antagonist in the pituitary. It binds to the receptors, and through apoptosis inhibition in lactotrophs, it increases PRL production (Gomes et al., 2009; Iwanaga et al., 2011; Radl et al., 2011). Additionally, in the experimental metoclopramide-induced model, a reduction in the synthesis of ovarian steroids during the proestrous phase, as well as a decrease in progesterone during pregnancy, has been observed (Betzold, 2004; Kinoshita et al., 2001). Histomorphometric analysis shows endometrial growth during diestrus (Panzan et al., 2006; Rossi et al., 2002), demonstrating the drug's interference in the reproductive system of the animals and its usefulness in studies of the disorder in vivo.

However, the real effects of both hyperprolactinemia and hormone replacement therapy on PRLR expression in the pituitary and the uterus are still unclear (Gomes et al., 2009). It is known that interference in the regulation of the expression of the receptor may change PRL action in target tissues. Such knowledge could aid in the choice of therapeutic strategies for women with hyperprolactinemia-induced infertility (Casanueva et al., 2006). Thus, the present study aims to analyze, in castrated adult female mice,

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0016-6480/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygcen.2013.04.037

the effect of metoclopramide-induced hyperprolactinemia on the expression of PRL and its receptors in the uterus and the pituitary as well as the effect of estrogen and/or progesterone therapy on the same expression.

2. Materials and methods

This research was approved by the local institutional committee. Number: 0413/09.

2.1. Animals

Sixty-four virgin female Swiss mice, each aged approximately 100 days and weighing on average 30 g, were confined to plastic cages measuring $45 \times 35 \times 15$ cm (length, width, and height, respectively) and having metal grid covers. Food and water were *ad libitum*. Room temperature was kept at 22 °C and light was provided artificially with fluorescent lamps (Phillips 40 W) in a photoperiodic cycle L12:D12 with light period 7 a.m.–7 p.m.

2.2. Surgery and animal treatment

Prior to oophorectomy, all animals underwent colpocytological exams for 3 weeks for characterization of the estrous cycle. A total of 12 animals with irregular cycles were excluded, and 3 died following castration. A total of 14 animals with intact ovaries were allocated to two groups: G1 (treated with saline solution) and G2 (treated with metoclopramide). A total of 35 oophorectomized animals was randomized (Iwanaga et al., 2011) to five subgroups of seven animals each: G3 (treated with saline solution), G4 (treated with metoclopramide), G5 (treated with metoclopramide + 17β-estradiol), G6 (treated with metoclopramide + micronized progesterone), and G7 (treated with metoclopramide + 17β-estradiol + micronized progesterone).

2.3. Solution administration

The drugs and the vehicle were administered subcutaneously using a volume of 100 μL (0.1 ml) per solution for 50 consecutive days. The daily metoclopramide (Sigma, St Louis, MO) dose was 200 μg in 0.9% saline solution. Micronized progesterone (Sigma) and 17β-estradiol (Sigma) were administered in oil-based solution in daily doses of 1 μg and 1 mg, respectively (Barañao et al., 1991; Rossi, 2009). At the end of the experimental period, one hour after solution administration, the animals were sacrificed in the proestrous phase, except for the oophorectomized animals, which continued to receive the solutions until they entered that phase. No animals were lost during the treatment phase.

2.4. Hormone measurements

Blood was collected for measurements of circulating concentrations of serum estradiol, progesterone, and PRL by radioimmunoassay (Siemens, Princeton, NJ – USA). Reactions were carried out in duplicate as previously described (Rossi et al., 2002).

2.5. Quantitative real-time polymerase chain reaction (qRT-PCR)

The uterus and the pituitary were removed and processed for total RNA extraction using the TRIzol (Life Technologies, USA) protocol. Synthesis of cDNA was performed from 2 μg of total RNA with the Hi Capacity Reverse Transcription Kit (Life Technologies, USA) following the manufacturer's instructions. The resultant cDNAs underwent conventional PCR using a pair of specific primers for the β-actin gene (forward – AAT TGT GGC TGA GGA CTT TG3′/reverse – CAC AGA AGC AAT GCT GTC AC) to verify synthesis effectiveness. Following fragment analysis in agarose gels (Invitrogen), the cDNAs were subjected to qRT-PCR reaction. Gene expression quantification was conducted using a RNA mixture of brains from normal female mice as a reference sample since they expressed both PRL and all of its isoforms in our tests. The oligonucleotide for amplification were the following: PRL (forward – AAT TAG CCA GGC CTA TCC TGA AG/reverse – GGA TGG AAG TTG TGA CCA AAC A), PRLR (forward – AAG CCA GAC CAT GGA TAC TGG AG), PRLR-L (reverse – AGC AGT TCT TCA GAC TTG CCC TT), PRLR-S1 (reverse – AAC TGG AGA ATA GAA CAC CAG AG), PRLR-S2 (reverse – TCA AGT TGC TCT TTG TTG TGA AC), PRLR-S3 (reverse – TTG TAT TTG CTT GGA GAG CCA GT), all designed with the Primer Express 3.0 (Applied Biosystems, Foster City, CA, USA) program and synthesized by Integrated DNA Technology (DNA Technologies, Coralville, IA, USA). Reactions were carried out in duplicate with the 7500 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) using a total volume of 25 μl with 450 nM of primers and SYBR Green PCR Master Mix (Applied Biosystems). The β-actin gene was selected as housekeeping gene for correction of variability. Calculations for transcript expression were made using the Pfafll method (Pfaffl, 2001).

2.6. Data analysis

The data were grouped by homogeneity in each treatment group for each specific result using the Kolmogorov–Smirnov (KS) test. Results were analyzed using the analysis of variance test, ANOVA, complemented by the Tukey–Kramer post hoc test (GraphPad Prism5), and they were expressed as average and standard deviation.

3. Results

Serum concentrations of estrogen, progesterone, and prolactin in the study groups: Estrogen concentration was higher in the two groups of non-castrated animals (G1 and G2) and in the two groups of animals treated with the hormone (G5 and G7). Progesterone also increased in G1 and G2 and in the groups of animals treated with the hormone (G6 and G7). As for PRL, an increase in serum level was observed in G2 and in the animals treated with progesterone in isolation (G6) or in association with estrogen (G7) Table 1.

3.1. Expression of PRLR and PRL in the pituitary

The PRLR in the pituitary showed that PRLR-S1, PRLR-S2, PRLR-S3, and PRLR-L were expressed in all animals in all groups. No differences in PRLR-S1 expression were found between the groups (Fig. 1B). No significant difference in PRLR-S2 expression was found

Table 1
Serum concentration of 17β estradiol, progestogen, and prolactin in the study groups.

	17β estradiol-pg/mL	Progestetone-ng/mL	Prolactin-ng/mL
G1	138.27 ± 3.58[a]	14.25 ± 3.15[b]	61.94 ± 2.12
G2	140.98 ± 5.82[a]	12.96 ± 1.33[b]	407.50 ± 5.52[c]
G3	3.32 ± 0.63	1.49 ± 0.10	58.48 ± 2.59
G4	5.29 ± 1.22	1.37 ± 0.27	204.91 ± 3.58
G5	141.04 ± 7.27[a]	0.85 ± 0.12	203.77 ± 4.97
G6	4.49 ± 1.23	14.07 ± 1.10[b]	335.73 ± 13.64[c]
G7	139.83 ± 4.10[a]	16.89 ± 0.31[b]	304.81 ± 4.79[c]

[a] higher concentrations of estrogen when compared to the OSS, OM, and OMP groups ($p < 0.05$).
[b] higher concentrations of progestogen when compared to the OSS, OM, and OME groups ($p < 0.05$).
[c] higher concentrations of prolactin when compared to the OSS group (ANOVA).

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