RAPID COMMUNICATION

Effects of metoclopramide on the mouse anterior pituitary during the estrous cycle

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INTRODUCTION

The symptoms of hyperprolactinemia in women mainly result from changes in the release of gonadotropins and the consequent repercussions on ovarian function.[1-3] Metoclopramide is a hyperprolactinemic drug that is used as an antiemetic agent.[4,5] In female mice, metoclopramide-induced hyperprolactinemia causes changes in the reproductive system, mainly in ovarian function and the endometrium.[6-10] While there is extensive data regarding prolactin regulation and hyperprolactinemic states, the direct effects of metoclopramide on the morphological and functional aspects of pituitary cells remain unknown. Therefore we have evaluated the histomorphological and immunohistochemical changes in lactotrophs of female mice upon treatment with metoclopramide.

MATERIALS AND METHODS

Eighty adult, virgin female mice of the Swiss EPM-1 strain were kept on food and water *ad libitum* at room temperature (22℃) and under artificial light with a photoperiod of 12 hours (lights on 7 a.m.–7 p.m.). The animals were randomly divided into 2 groups of 40 animals each: control (Ctr), which were injected daily with drug vehicle (0.2 ml of saline solution), and experimental (HPrl), treated with metoclopramide (6.7 μg/g daily).[6-10] Injections were made by the subcutaneous route at 11 a.m., and the treatments lasted 50 days. The experimental protocol was approved (Report No. 0809/04) by the UNIFESP-EPM Review Board.

On day 50, 1 h after the last injection, colpocytological examination of vaginal smears was carried out for all animals, which were in each of the 4 phases of the estrous cycle (10 mice per phase in both Ctr and HPrl groups), before they were euthanized by decapitation. The animals not typified remained on treatment and were euthanized once their phase was diagnosed. Hormone levels were measured in blood samples taken immediately after euthanasia. Pituitary glands were carefully dissected out and immediately immersed in Bouin's liquid for further histological processing.

Serum 17β-estradiol and progesterone was determined using their respective kits (ICN Biomedicals Inc., Costa Mesa, CA, USA). The samples were assayed in duplicate and processed on the same day. The detection limits for estradiol and progesterone were approximately 0.1 pg/ml and 0.15 ng/ml, respectively. Crossreactivity with other steroids for both assays was less than 0.01%. Serum prolactin (PRL) was determined using a mouse PRL radioimmunoassay kit.

For immunohistochemistry analyses, pituitaries were fixed in Bouin's fluid for 6 hours and embedded in paraffin; 5-μm sections were microwaved in 1 M sodium citrate buffer (pH 6.0) for 8 min in order to retrieve the antigen.[11] After treatment with 0.1% Triton X-100 for 10 min, endogenous peroxydase activity was blocked with 0.3% H_2O_2 in methanol for 30 min. Antibody non-specific binding was blocked with normal goat serum (VECTASTAIN Elite ABC kit, Vector Laboratories) for 20 min at room temperature. The sections were then incubated overnight with primary antibody at 4–8℃ (1:600, A0569, Dako Cytomation). After washing in PBS, sections were incubated with the secondary antibody, rabbit anti-chicken IgG (1:800, DakoCytomation) for 15 min. Reaction products were visualized by using 3,3'-diaminobenzidine (DAB, Sigma Chemical Co). The negative control was prepared by incubating sections with rabbit immunoglobulin fraction (DAKO Cytomation) instead of the primary antibody. Sections were counterstained with hematoxylin.

The number and the nuclear volume of prolactin-immunolabeled cells (lactotroph cells) were evaluated in images taken by a high-resolution camera (AxioCam MRC, Carl Zeiss) at a final magnification of x400. The camera was connected to a computer coupled to a light microscope (Axiolab Standard 2.0, Carl Zeiss) and images were analyzed using REL AxionVision 4.6 software (Carl Zeiss). To evaluate the number of lactotroph cells, 8 fields were captured for every sample with a x40 objective (37,745.55 μm^2 field area) and a total of 80 fields were analyzed for each group (total area for group = 299,644.00 μm^2). To calculate nuclear volume, the largest (a) and the shortest (b) nuclear diameters from 50 lactotroph cells for every animal were measured at a final magnification of x1000 and then used in the formula $V = a.b^2/1{,}91$.[12] The results were analyzed by analysis of variance (ANOVA) followed by the Kruskal-Wallis test ($p < 0.05$).

RESULTS

The level of 17β-estradiol was higher in the control (Ctr) group than in the HPrl group in all phases, particularly

Table 1 - Circulating hormones and pituitary morphometric data throughout the estrous cycle in female control mice (Ctr) or mice rendered hyperprolactinemic (HPrl) by treatment with metoclopramide.

	Proestrus		Estrus		Metaestrus		Diestrus	
	Ctr	HPrl	Ctr	HPrl	Ctr	HPrl	Ctr	HPrl
Serum hormones:								
17β-Estradiol (pg/mL)	121.2 ± 8.2^{a}	90.3 ± 3.9^{b}	92.3 ± 2.8	45.3 ± 3.1^{b}	65.8 ± 5.7	55.3 ± 2.8^{b}	72.4 ± 3.5	59.3 ± 4.2^{b}
Progesterone (ng/mL)	11.6 ± 0.5^{a}	3.7 ± 0.1^{b}	5.7 ± 0.4	2.7 ± 0.2^{b}	6.1 ± 0.1	1.9 ± 0.4^{b}	7.7 ± 0.2	2.9 ± 0.3^{b}
Prolactin (ng/mL)	44.1 ± 7.1^{a}	301.2 ± 42.1^{b}	28.8 ± 8.1	274.3 ± 35.5^{b}	20.8 ± 5.3	264.1 ± 42.9^{b}	19.3 ± 5.6	269.1 ± 42.1^{b}
Pituitary lactotrophs:								
Density (cells/1000 μm^2)	9.1 ± 0.3	12.2 ± 0.1^{b}	8.4 ± 0.1	10.2 ± 0.2^{b}	8.0 ± 0.2	11.0 ± 0.1^{b}	$8.1 \pm 0,3$	9.7 ± 0.2^{b}
Cell volume (μm^3)	$151,33 \pm 7.1^{a}$	176.2 ± 9.1^{b}	$128,58 \pm 7.1$	169.3 ± 8.5^{b}	102.48 ± 5.3	166.1 ± 8.9^{b}	106.1 ± 4.6	165.1 ± 8.1^{b}

Data are shown as mean $\pm$ SD.
[a]indicates $p < 0.05$ when compared to other phases of the Ctr groups.
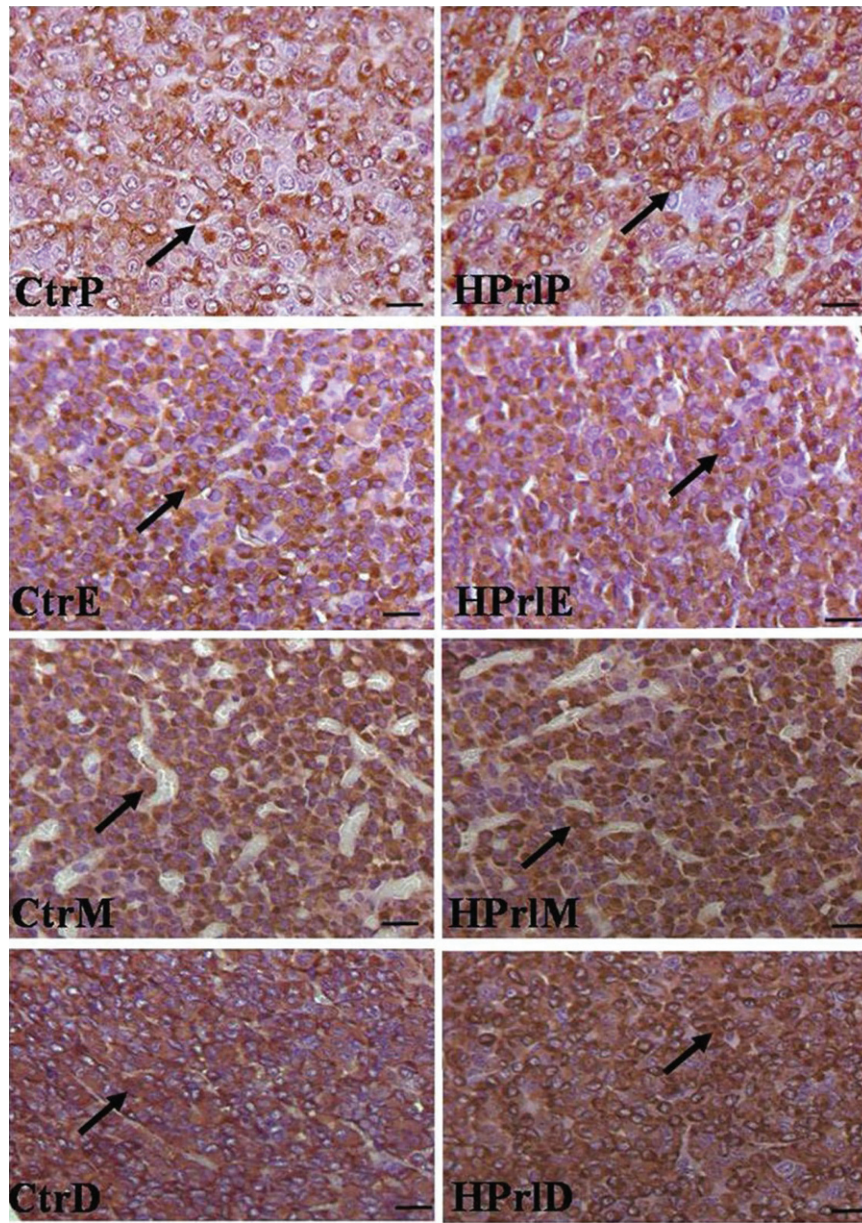[b]indicates $p < 0.05$ when compared to the Ctr group in each estrous cycle phase.

Fig. 1 - Immunohistochemical localization of prolactin in adenohypophyseal lactotrophs in female control (Ctr) mice or mice treated with metoclopramide (HPrl) in the estrous phase. Arrows indicate prolactin-positive cells. P = Proestrus; E = Estrus, M = Metaestrus; D = Diestrus. Scale bar = 20 μm.

during the proestrous phase (Table 1). However, the level of progesterone was higher in the Ctr group (Table 1). Also, the highest level of prolactin in the HPrl group occurred during the proestrus phase (Table 1). PRL immunolabeling of pituitary glands revealed markedly stronger expression in the metoclopramide group (Fig. 1). Metoclopramide-treated female mice had significant increases in the number and the volume of lactotroph cells of the pituitary gland during all phases of the estrous cycle (Table 1).

DISCUSSION

In the neuroendocrine axis, dysfunction of hypothalamic dopamine or its pituitary receptors leads to hyperprolacti-nemia and reproductive disturbances.[13,14] The PRL secretory profile is well established in female mice; circulating PRL remains low in normal cycling animals throughout most of the estrous cycle. There is one prominent PRL surge at proestrus, which is coincident with luteinizing hormone (LH), for a period of 4–5 days.[3] Our serum PRL results also revealed higher levels in Ctr proestrus animals than control rats in other phases.

Metoclopramide has been used to study the effects of the elevation of prolactin (PRL) plasma levels on the body for many years[15]. However, the pituitary synthesizes and secretes several distinct hormones. Therefore, we employed an immunohistochemical PRL marker to identify lactotroph cells.[16]

We found that metoclopramide not only increased the number but also stimulated the metabolic activity of lactotrophs, as indicated by the enlarged nuclear volumes in hyperprolactinemic animals irrespective to the phase of the estrous cycle. Given the existence of a definite relation-ship between nuclear volume and cell metabolism,[12] it is conceivable that the observed increase of circulating PRL is due to an increase of the number and the activity of lactotrophs. Interestingly, metoclopramide-treated animals showed high levels of circulating PRL and increased number and nuclear volumes of lactotrophs, and this profile did not change throughout the phases of the estrous cycle.

Dopamine and its agonists regulate many systems through stimulation of adrenergic and dopaminergic recep-tors and also regulate PRL production.[17] As a D2 receptor antagonist, metoclopramide affects PRL secretion.[7,8,13,18] The long and short isoforms of the dopamine receptor (D2L and D2S) exhibit similar pharmacological properties and are co-expressed in the same cells.[19] The D2L isoform is predominant in the rat pituitary, striatum, and olfactory tubercle, whereas D2S is more abundant in the hypothala-mus.[20-22] Activation of either isoform in rat lactotrophs mediates dopamine suppression of the PRL gene.[23]

While PRL directly inhibits secretion of gonadotropins from the anterior pituitary, there is accumulating evidence that PRL also exerts a direct inhibitory effect on gonado-tropin actions in the ovary[24]. Physiological levels of PRL suppress follicle-stimulating hormone (FSH)-induced estra-diol production through a reduction in aromatase expres-sion and PRL increases progesterone production by augmenting StAR, P450scc, and 3βHSD expression in granulosa cells.[25] However, hyprolactinemia may inhibit GnRH release and reduce ovulation, explaining the reduc-tion in progesterone.[7,8,24] Also, metoclopramide-induced hyperprolactinemia correlates with a low number of luteal bodies, which are ovarian structures responsible for progesterone production.[7]

The central PRL-secretion regulator dopamine inhibits lactotroph proliferation and decreases the size of hyper-trophied lactotrophs.[26,27] Our results show that daily treatment with metoclopramide induces a consistent hyper-prolactinemic state and increases the number and the volume of lactotrophs. Thus, our data suggest that besides the increase of pituitary PRL secretion, significant prolifera-tion of the pituitary gland occurs. If this process were to continue, we would predict an evolution towards pituitary gland tumorigenesis. We also observed a significant decrease in progesterone levels coexisted with PRL serum elevation. Overall, these disturbances may have deleterious effects on ovarian function,[7] endometrial tissue,[9] and embryo implantation.[8] Finally, hyperprolactinemia caused by metoclopramide in mice is due to an increase in the number and activity of lactotrophs and these alterations seem to be independent of the cycle phase.

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