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Antisense mRNA for NPY-Y_1 receptor in the medial preoptic area increases prolactin secretion

N.A. Silveira[1] and C.R. Franci[2]

[1]Departamento de Fisiologia, Instituto de Ciências Biológicas, Universidade de Goiás, Goiânia, GO, Brasil
[2]Departamento de Fisiologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

Correspondence
C.R. Franci
Departamento de Fisiologia
Faculdade de Medicina de
Ribeirão Preto, USP
Av. Bandeirantes, 3900
14049-900 Ribeirão Preto, SP
Brasil
Fax: +55-16-633-0017
E-mail: crfranci@fmrp.usp.br

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Abstract

We investigated the participation of neuropeptide Y-Y_1 receptors within the medial preoptic area in luteinizing hormone, follicle-stimulating hormone and prolactin release. Four bilateral microinjections of sense (control) or antisense 18-base oligonucleotides of messenger ribonucleic acid (mRNA) (250 ng) corresponding to the NH_2-terminus of the neuropeptide Y_1 receptor were performed at 12-h intervals for two days into the medial preoptic area of ovariectomized Wistar rats (N = 16), weighing 180 to 200 g, treated with estrogen (50 µg) and progesterone (25 mg) two days before the experiments between 8.00 and 10:00 a.m. Blockade of Y_1 receptor synthesis in the medial preoptic area by the antisense mRNA did not change plasma luteinizing hormone or follicle-stimulating hormone but did increase prolactin from 19.6 ± 5.9 ng/ml in the sense group to 52.9 ± 9.6 ng/ml in the antisense group. The plasma hormones were measured by radioimmunoassay and the values are reported as mean ± SEM. These data suggest that endogenous neuropeptide Y in the medial preoptic area has an inhibitory action on prolactin secretion through Y_1 receptors.

Key words
- Neuropeptide Y
- Medial preoptic area
- Neuropeptide Y-Y_1 receptor
- Prolactin
- mRNA for neuropeptide Y-Y_1 receptor
- Luteinizing hormone
- Follicle-stimulating hormone

Introduction

Neuropeptide Y (NPY) is a single chain containing 36 amino acids. It was first isolated from porcine brain and is widely distributed among neurons of the central and peripheral nervous systems. A high density of cell bodies occurs in the arcuate nucleus of the hypothalamus, projecting to various brain regions such as the lateral septum, medial preoptic area (MPOA), paraventricular nucleus, and solitary tract nucleus (1-3). The MPOA contains cell bodies, a moderately high density of terminals with NPY and extensive networks of NPY-positive fibers (4-6). It has been shown that NPY receptors of subtypes Y_1 and Y_2 appear to occur in the MPOA (7,8). In rats, the MPOA is an important region for brain sexual differentiation during fetal and neonatal life and participates in the cyclic secretion of gonadotropins in females starting from puberty (9,10).

Intracerebroventricular microinjection of NPY increases plasma luteinizing hormone (LH) in ovariectomized rats (11). NPY has been shown to have a stimulatory action on the preovulatory LH peak of proestrus in normal rats and on the peak induced by

ovarian steroids in ovariectomized rats (12). The control of prolactin (PRL) secretion depends on various inhibitory and stimulatory hypothalamic factors which, taken together, produce an inhibitory tonus for PRL secretion (13). Dopamine (DA) secreted by neurones of the tuberoinfundibular system has been considered to be the most significant inhibitory factor. Intracerebroventricular (*icv*) NPY administration inhibits PRL secretion in males partly by increasing DA secretion (14-16). Intracerebroventricular administration of NPY increased the activity of tuberoinfundibular dopaminergic neurones in males (17). However, central administration of anti-NPY antibody did not promote an increase in plasma PRL (18).

The purpose of the present study was to determine the role of neuropeptide Y_1 receptors within the MPOA on follicle-stimulating hormone (FSH), LH and PRL secretion.

Material and Methods

Female Wistar rats weighing 180 to 200 g were maintained under controlled conditions of temperature (22-24°C), humidity and light (12-h light/12-h dark), with free access to water and ration. The animals were submitted to bilateral ovariectomy and two weeks later bilateral stainless steel cannulae were implanted into the MPOA with the aid of a KOPF stereotaxic apparatus according to the following coordinates: AP 2.0 mm anterior to the bregma; L 1.0 mm from the midline; V 7.5 mm below the top of the skull. The cannula was fixed with self-polymerizing acrylic resin (Simplex Dental-DFL, Rio de Janeiro, RJ, Brazil) and its lumen was occluded with a stainless steel mandril. Both surgeries were performed under sodium thiopental anesthesia (Abbott Laboratories, Chicago, IL, USA; 50 mg/kg, *ip*). An antibiotic (Pentabiótico Veterinário, Wyeth-Ayerst, Marietta, PA, USA; 0.2 ml/rat) was injected intramuscularly after both surgeries.

The animals received a subcutaneous transmuscular injection of estradiol benzoate (Schering, Kenilworth, NJ, USA; 50 µg) and progesterone (Sigma Chemical Co., St. Louis, MO, USA; 25 mg) in corn oil (0.5 ml) two days before the experiment between 8:00 and 10:00 a.m.

During two days before the experiments, four bilateral microinjections were performed into the MPOA at 12-h intervals for the administration of mRNA sense (5'AATTC AACTCTGTTCTCC-3') or antisense (5'GG AGAACAGAGTTGAATT3') oligonucleotides for NPY-Y_1 receptors at the dose of 250 ng in 1 µl saline. This base sequence was the same as that previously utilized for the specific reduction of NPY-Y_1 receptor sites (19). The oligonucleotides were synthesized in the Hematology Laboratory of the University Hospital, Medical School of Ribeirão Preto, under the supervision of Dr. Marco A. Zago.

On the day of the experiment, approximately 12 h after the last oligonucleotide microinjection, the animals were sacrificed by decapitation. Blood was collected and the brains were removed and fixed in formalin for histological analysis. Only values for animals with the cannula positioned in the MPOA were used for analysis.

Blood samples were centrifuged at 2500 rpm for 15 min and the plasma was separated and frozen at -20°C until the time for measurement of LH, FSH and PRL by RIA using National Institute of Arthritis and Digestive Diseases and Kidney (NIADDK) kits. The results are reported as RP-3 standard reference for LH and PRL and as RP-2 for FSH. The intra-assay coefficient of variation was 3.4% for LH, 2.5% for FSH and 2.3% for PRL.

The significance of the differences between groups was analyzed by the unpaired Student *t*-test.

Results and Discussion

The results are illustrated in Figure 1.

After four microinjections of antisense mRNA oligonucleotide for NPY-Y_1 receptors into the MPOA, plasma LH and FSH levels were similar to those for the control group microinjected with sense oligonucleotide. However, plasma PRL was significantly higher in the antisense oligonucleotide group than in the sense oligonucleotide group ($P<0.02$). Thus, the antisense mRNA oligonucleotide for Y_1 receptors increased the plasma PRL but did not change plasma LH or FSH.

Intracerebroventricular microinjection of NPY at the doses of 235 and 470 pmol (11) or intracardiac administration of anti-NPY serum (20) induced an increase in plasma LH in ovariectomized rats. NPY has been shown to stimulate the LH preovulatory peak of proestrus in normal rats and the peak induced by ovarian steroids in ovariectomized rats (12). This peak induced in ovariectomized rats by administration of estrogen for two days and of progesterone on the third day was blocked by immunoneutralization with anti-NPY serum (21) or microinjection of antisense mRNA oligonucleotide for NPY into the lateral ventricle (22). However, there was no difference in plasma LH from ovariectomized rats treated with a simultaneous injection of estrogen and progesterone for 48 h that received an *icv* microinjection of 470 pmol of NPY or saline (23). We also used ovariectomized rats treated with a simultaneous injection of estrogen and progesterone 48 h before the experiment but we did microinject antisense mRNA oligonucleotide for NPY_1 into the MPOA. This structure is very important in the control of cyclic gonadotropin secretion in rats (9). In our studies, the control of LH and FSH secretion was not influenced by NPY-Y_1 receptors within the MPOA. However, we cannot rule out the possibility that NPY may act through Y_1 receptors in the MPOA to control LH secretion in other situations such as proestrus or the induced peak. Furthermore, a possible action of endogenous NPY through Y_1 receptors to control gonadotropin secretion may not occur at the MPOA level but rather at the level of other brain structures.

There are few data about the effects of NPY on PRL secretion. Intracerebroventricular microinjection of NPY inhibited PRL secretion in male rats (22). Our work showed that plasma PRL increased in female rats submitted to treatment with antisense mRNA oligonucleotide for Y_1 receptors.

Inhibitory and excitatory actions by the MPOA on PRL secretion have been reported in different situations. Electrical stimulation of the MPOA increased PRL secretion in males and decreased it in proestrus females but had no effect on diestrous females (24). MPOA possesses inhibitory and stimulatory neurons for nocturnal and diurnal surges of PRL, respectively (25). Studies of fos expression patterns during the estrous cycle showed that MPOA may be an important site for the integration of stimuli associated with the proestrous PRL surge (26) which is abolished by ovariectomy. On the other hand, estradiol implantation into the MPOA but not into the cerebral cortex induces a PRL surge in ovariectomized rats (27). Microinjection of substance P into the MPOA increased PRL secretion in male rats (28) while microinjection of angiotensin II into the same area inhibited PRL secretion in estrogen-primed ovariectomized rats. The latter response was blocked by previous microinjection of losartan (29,30).

The inhibitory action of the MPOA on PRL secretion may utilize NPY as a neuro-

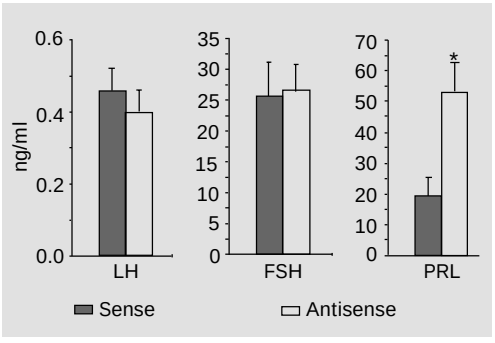

Figure 1 - Effect of four microinjections of sense or antisense mRNA oligonucleotides (250 ng) for neuropeptide Y-Y_1 receptors into the medial preoptic area at 12-h intervals on luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin (PRL) secretion in ovariectomized rats treated with estrogen (50 µg) and progesterone (25 mg). Values are reported as means ± SEM for 8 animals. *$P<0.02$ *vs* sense group (control).

mediator through Y_1 receptors. However, we cannot rule out other neuromediators although the interaction of the inhibitory actions of NPY in the MPOA and other inhibitory factors is unknown and should be investigated in future studies.

In conclusion, the deficiency of NPY-Y_1 receptors prevented the inhibitory action of endogenous NPY on PRL secretion and caused an increase of this secretion. Our results confirm the inhibitory action of NPY on PRL secretion also in ovariectomized rats treated with estrogen and progesterone. Furthermore, they show that the effect occurs through Y_1 receptors and that one of the sites for this action is the MPOA.

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