PEPTIDES 27 (2006) 2295–2299

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

Effect of alpha-melanotropin hormone on serum levels of luteinizing hormone and progesterone in experimental rat autoimmune oophoritis

Victoria Berberian [a], Silvina Sánchez [a], Mariela Sánchez-Borzone [a], Andrés M. Attademo [a], Mercedes Lasaga [b], Maria Ester Celis [a,*]

[a] Laboratorio de Ciencias Fisiológicas, Departamento de Farmacología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Argentina

[b] Centro de Investigaciones en Reproducción, Facultad de Medicina, Universidad de Buenos Aires, Piso 10, Buenos Aires C 1112 ABG, Argentina

ARTICLE INFO

Article history:
Received 8 March 2006
Accepted 8 March 2006
Published on line 22 May 2006

Keywords:
Experimental autoimmune oophoritis
Hormones
α-MSH effect
Inflammatory process
icv injections

ABSTRACT

We studied the effect of alpha-melanotropin hormone (α-MSH) on experimental autoimmune oophoritis (EAO), an inflammatory process induced in female rats. During proestrus, serum levels of LH and progesterone in rats with EAO were higher than those of control rats. However, administration of α-MSH to these rats decreased the levels of LH. Similarly, in the following diestrus, rats with EAO had high levels of LH but treatment with α-MSH decreased the levels to diestrus 2 control values. Treatment with α-MSH also reduced the LH levels of control rats in diestrus 2 compared to untreated controls. However, α-MSH treatment had no effect on progesterone levels of either control or rats with EAO. Thus, although α-MSH induced notable changes in levels of LH, this decrease was unable to block the illness.

1. Introduction

It is believed that infertility and ovarian disorders in women are caused not only by alterations in the immune function but also by changes in the reproductive hormones. Furthermore, the prevalence of these diseases might be the consequence of a bi-directional signalling network between the immune and the endocrine systems. There is a strong analogy between experimental autoimmune oophoritis (EAO) in rats and human inflammatory disease, a localized response to tissue injury. These types of inflammatory disorders are usually difficult clinical situations.

One of the mediators of inflammation is the endogenous neuropeptide α-melanotropin (α-MSH), a basic tridecapeptide derived from the precursor molecule POMC. This peptide is found mainly in the pituitary gland but also in low concentrations in other areas of the central nervous system as well as in skin, placenta, testes and ovaries. α-MSH is an important modulator of host reactions including fever and inflammation [5,8,17,18,23]. It is also involved in other functions, such as memory and attention [2,20], sexual and grooming behaviors [13,22,25] and reproduction [3,10,21]. Our laboratory previously reported that α-MSH can modify the levels of both progesterone and nitric oxide in cultured ovarian granulose cells obtained at different stages of EAO [4].

* *Corresponding author*. Tel.: +54 351 4332021; fax: +54 351 4332020.
E-mail address: mefisiol@mater.fcm.unc.edu.ar (M.E. Celis).
0196-9781/$ – see front matter
doi:10.1016/j.peptides.2006.03.001

Considering the anti-inflammatory faculties of α-MSH and its influence on ovarian function [11], the aim of the present work was to study whether α-MSH is capable of blocking the induced illness and/or of modifying serum levels of LH and progesterone.

2. Materials and methods

2.1. Animals

Female Wistar rats weighing 200–250 g were housed and reared at the Laboratory of Physiological Sciences, Department of Pharmacology, Faculty of Medical Sciences of the National University of Córdoba, Argentina, according to this institution's guidelines of Animal Care. The rats were kept in controlled conditions of light (lights on 06:00–20:00 h) and temperature (22 ± 1 °C) with food and water freely available. Daily vaginal smears were examined, and rats with two or more consecutive 4-day estrous cycles were used for experimental purposes.

2.2. Immunization and histology

Animals immunizing using the modified Damjanovic and Jancovic [9] model. Briefly, five to six fresh rat ovaries were homogenized with complete Freud adjuvant (CFA). The ovary-adjuvant mixture was injected into the footpads of both rear legs (100 mg of fresh tissue/0.25 ml/rat); control rats were injected only with the adjuvant and their behavior was similar to that of untreated rats. Immunized animals exhibited constant diestrus between days 7 ± 1 to 14 ± 1 and were used only during the following first proestrus and in the middle of the constant diestrus. Control rats were used during proestrus and diestrus 2.

The ovaries were fixed in Carnoy's fluid and processed for staining with haematoxylin and eosin (H:E). The histopathological examination of the ovaries showed the presence of inflammatory mononuclear infiltrates in the interfollicular connective tissue, near the blood vessels. These infiltrates consisted predominantly of lymphocytes, a few plasma cells and some histiocytes. Control rats showed a normal appearance of the ovaries. Only rats with this characteristic were considered with EAO. These results indicated an inflammatory process. And in all these rats, it is possible to observe 7 days of continuous diestrus. All the rats that not shown these manifestations were discarded.

2.3. Intracerebroventricular cannulation

Rats were anesthetized with pentobarbital (30 mg/kg i.p.) and placed on a stereotaxic frame. A stainless steel cannula (15 mm long, 0.65 mm o.d.) was implanted intracerebroventricularly (icv) into the third ventricle at the appropriate rostral/caudal (vertical: 0.42, anteroposterior: 0.30 and lateral: 0.0) [16]. The cannula was cemented into place with dental acrylic and the external parts were sealed with a piece of plastic tubing. After surgery the animals were individually housed and given food and water ad libitum. At the end of the experiment, rats were killed and the brains fixed in a 4% formalin solution. After freezing, 120 μm slices were obtained for histological examination to confirm the correct position of the cannula. Animals with misplaced cannulas were excluded from the analysis.

2.4. Blood samples

A polyethylene cannula was placed in the jugular vein of rats anesthetized with pentobarbital (30 mg/kg i.p.). The exterior end of the cannula was guided under the skin until it emerged at the back of the head and was then sutured in place. This procedure was completed before 8:00 h on the blood-sampling day.

2.5. Effect of α-MSH on the serum levels of LH and progesterone in proestrous and diestrous rats

The effect of α-MSH on serum levels of LH and progesterone was observed in both proestrous and diestrous rats. Each experimental animal received daily injections of 1 μg/μl α-MSH or 1 μl ACSF (controls) at 12:00 and 14:00 h during the study. On the first day of the experiment, all the rats were injected with CFA at 13:00 h. Blood samples were taken every hour between 12:00 and 20:00 h. The plasma was then separated by centrifugation at 1085 × *g* for 15 min and stored at −20 °C until assayed. The erythrocytes were re-suspended in saline solution and infused after the blood sample.

2.6. Effect of α-MSH on the serum levels of LH and progesterone in proestrous and diestrous rats with EAO

Rats were injected with 1 μg/μl α-MSH or 1 μl ACSF (controls) at 12:00 and 14:00 h throughout the study period. EAO was induced on day 1. Blood samples were taken every hour as previously described for control rats.

2.7. Progesterone radioimmunoassay

The serum concentration of progesterone was measured by direct RIA (ICN Biomedicals, Thame, Oxfordshire, UK). Inter- and intra-assay coefficients of variation were below 10%. The cross reaction with androstenedione was 0.23%.

2.8. LH radioimmunoassay

Serum LH levels were measured by a double-antibody radioimmunoassay with reagents provided by Dr. A.F. Parlow (NIDDK). Rat LH-RP-3 was used as the standard. Cross-reactivity with other pituitary hormones was negligible. Sensitivity of the LH radioimmunoassay was 0.1 ng/ml. The intra and inter-assay coefficients of variation were under 10%. LH concentration was expressed as ng/ml.

2.9. Statistical analysis

Data are expressed as the mean ± S.E.M. Differences between the groups were determined by ANOVA followed by LSD post hoc test. $P < 0.05$ was considered significant. The