Biochemical and Biophysical Research Communications 478 (2016) 999–1005

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Changes in progesterone receptor isoforms expression and in the morphology of the oviduct magnum of mature laying and aged nonlaying hens

María Genoveva González-Morán

Facultad de Ciencias, Laboratorio de Biología de la Reproducción Animal, Departamento de Biología Comparada, Universidad Nacional Autónoma de México, C.U., Av. Universidad 3000, Coyoacán, Ciudad de México, C.P.04510, Mexico

ARTICLE INFO

Article history:
Received 28 July 2016
Accepted 11 August 2016
Available online 12 August 2016

Keywords:
Oviduct
Progesterone receptors
Aging
Immunolocalization

ABSTRACT

The aim of this study was to evaluate changes in the morphology and expression of progesterone receptor (PR) isoforms in different cell subpopulations of the magnum region of the left oviduct of mature laying and aged nonlaying hens through histomorphometric and immunohistological methods. Histological results demonstrated several changes in the oviduct magnum of mature and aged hens, mainly in the mucosal tissue. Immunohistochemical analysis showed that both PR isoforms are expressed in all cell types of the oviduct magnum of mature laying hens. In contrast, in each cell type of the oviduct magnum of aged nonlaying hens only one PR isoform (PR-A or PR-B) was expressed. The results indicate that PR percentage and the PR-A and PR-B ratio change according to the cell type of the oviduct magnum and in an age-specific manner, and suggest that these variations contribute to the regulation of progesterone actions in the oviduct magnum with the normal aging of the animal.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

The left oviduct of the sexually mature hen is divided in five regions: infundibulum, magnum, isthmus, uterus, and vagina. The infundibulum forms a strong perivitelline membrane and chalaza around the egg yolk, the magnum is responsible for the synthesis and secretion of albumen, the isthmus forms a fibrous membrane around the egg white, the uterus forms the egg shell, and finally the vagina connects the uterus to the cloaca [1,2]. Therefore, the oviduct plays a vital role in the assembly of egg components after receiving ova from the ovary. This organ is fully developed only on the left side in most adult birds [3].

Most histological studies have been made in the oviduct of sexually mature domestic birds [1,2,4,5] and in some embryonic and post-hatching stages [6], but there are no histomorphometric studies in the oviduct of aged nonlaying hens.

It is known that the sex steroid hormones, progesterone and estrogen, play a role in the regulation of secretory processes in the oviduct [7–9]. Most of these processes are mediated by specific intracellular receptors, which are ligand-dependent transcription factors that regulate gene expression [10,11].

Chick progesterone receptors (PR) are expressed as two isoforms: PR-A (79 000 Da) and PR-B (110 000 Da) [12,13]. PR-A is an N-terminally truncate form of PR-B. The PR isoforms are derived from a single gene and generated from alternative translational start sites in the chick [14]. It has been shown that the PR isoforms regulate different genes and exert distinct functions [15]. In the adult chick oviduct, an approximately equimolar expression of PR-A and PR-B is observed [16], but in the estrogen-withdrawn chick and the aged nonlaying hen, when the oviduct is not biologically active, PR-A is the predominant isoform [17,18]. There is a diminution in PR-A isoform expression during seasonally changes in the oviduct. Thus, PR-A diminishes during winter and increases its expression during spring [19].

Most studies on PR isoforms localization in the chicken oviduct are restricted to immature chick oviduct after estrogen or progesterone treatment [20–22], as well as to the chick embryo and some post-hatching stage [6,23–25].

However, it is not known which cellular types express PR isoforms in the magnum of the oviduct of mature laying and aged nonlaying hens. For this reason, the purpose of the present study was to determine histological changes and the localization of PR isoforms in different cell populations from the magnum portion of the oviduct of mature laying and aged nonlaying hens through

E-mail address: bita@live.com.mx.

http://dx.doi.org/10.1016/j.bbrc.2016.08.071
0006-291X/© 2016 Elsevier Inc. All rights reserved.

histomorphometric methods and immunohistochemistry analysis.

2. Materials and methods

2.1. Animals

Laying White Leghorn hens (approximately 10–12 months-old, in production and laying five o more eggs in a sequence), and aged hens or nonlaying hens,"out of production" (approximately 48–52 months-old, cessation of reproductive function) were kept in individual cages under a 14:10 h light-dark cycle (lights on 6:00–20.00), with food and water *ad libitum*. Animals, eight per group (mature laying and aged nonlaying hens), were killed by decapitation, and the whole left oviduct was excised immediately and cleaned from adhesive tissue, recording its wet weight, length, and individual magnum's length.

Fragments of cephalic region of the oviduct magnum were fixed for histomorphometric and immunohistochemical studies. Experiments were performed according to the Official Mexican Norm (NOM-062-ZOO-1999) in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health of the USA, to ensure compliance with established international regulations and guidelines.

2.1.1. Histomorphometric evaluations

Fragments of cephalic region of the oviduct magnum were fixed in 4% paraformaldyde for 4 h, dehydrated and embedded in paraplast. Three transverse sections per animal (8 animals per group) of 5 μm in thickness were stained with hematoxylin-eosin for histological and morphometric evaluations under light microscopy.

Morphometric analyses were performed in some restricted magnum areas: luminal, tunica mucosal (consisting of mucosal epithelium and lamina propia-submucosa), and tunica muscularis. Total magnum wall area corresponded to the sum of all areas. The number of cells in the magnum's mucosal epithelium area was determined in four randomly selected regions for each section per animal.

All areas were digitalized by using the Image-Pro plus 6 image analysis program. Epithelium thickness was measured every 75 μm at high magnifications using an ocular micrometer, across the whole epithelial area in each section.

2.2. Immunohistochemistry

Oviduct magnum sections of 5-μm thickness were mounted on slides coated with poly-L-lysine (Sigma, St, Louis, MO, USA). They were deparaffinized, rehydrated through graded concentrations of alcohol to distilled water. The slides were incubated in a microwave oven with 10 mM citric acid, pH 6.0, for two cycles at 750 for 10 min each. Ten minutes were left between cycles. Then, slides were washed twice with 0.05 M sodium phosphate buffer (PBS) at pH 7.4. The microwave oven pretreatment procedure is a method used for the recovery of antigens and to unmask the epitope to which the monoclonal antibody binds [26]. Then, the slides were incubated in 3% hydrogen peroxide for 10 min, 0.5% Triton X-100 in PBS for 20 min; 1% normal swine serum in PBS for 20 min, and monoclonal antibodies PgR Ab-8 (clone hPRa2+hPRa3) or PgR Ab-6 (clone hPRa6) (NeoMarkers, Fremont, CA, USA), the former recognizes both PR isoforms whereas the latter only recognizes the PR-B isoform (4 μg/ml) in PBS containing 0.3% Triton X-100 and 0.1% gelatin during 48 h, at 4 °C in a humid chamber. Sections were incubated with biotinylated secondary antibody for 30 min at room temperature, and then with streptavidin-peroxidase conjugated for 30 min. Sections were washed twice with PBS between incubations. Peroxidase activity was revealed with 3, 3′diaminobenzidine chromogen solution in the presence of hydrogen peroxide. After washing, sections were dehydrated and mounted with Canada Balsam without counterstaining. Immunohistochemical negative controls consisted of adjacent sections incubated with preimmune serum instead of the primary antibody.

Total number of positive PR isoforms immunostained cells was determined in four scattered representative fields of each section in: mucosal epithelium cells (epithelial basal and epithelial apical cells), glandular epithelial cells, stromal cells, muscle cells and serosa cells of the cephalic region of the oviduct magnum per each section (3 sections per animal) in 8 oviducts per group (mature laying and aged nonlaying hens). Immunopositive cells were considered those with brown staining of the nucleus.

Because there are no antibodies that recognize PR-A, the immunohistochemical analysis of PR-A isoform was performed by subtractive inference between the number of immunoreactive cells incubated with PgR Ab-8, which recognizes both isoforms (PRAB), minus the number of immunoreactive ones incubated with PgR Ab-6 that only recognizes PR-B.

2.2.1. Statistical analysis

Student's *t*-test was used for statistical evaluation.

3. Results

3.1. Macroscopical observations and oviduct dimensions

In the mature laying hen the oviduct is a tortuous tube, extending from the left ovary to the cloaca and occupying a large part of the abdominal cavity, but in aged nonlaying hen it is a flat and thin tube (Fig. 1A–D).

Data revealed that in the mature laying hen, mean weight and length of the oviduct were significantly bigger than in the aged nonlaying hen (Table 1), but still retaining its five segments (infundibulum, magnum, isthmus, uterus and vagina). The oviduct magnum is the longest of the total oviduct length in laying and aged hens (Fig. 1B and C) (Table 1).

3.1.1. Histological and morphometric analysis

Histological observations showed that the wall of the magnum of the mature laying and aged nonlaying hens consists of three tunicas: mucosal, muscularis and serosa (Fig. 1E and F). Tunica mucosal forms convolutions that protrude into the lumen. This mucosal tissue consisted of the surface epithelium (mucosal epithelium) and the lamina propia-submucosa, containing tubular glands and loose connective tissue (Fig. 1E–H). In the mature laying hen the mucosal epithelium was cuboidal with ciliated cells (Fig. 1G and I), and mucosal folds are prominent; in consequence, the magnum lumen is reduced to narrow clefts between them, the folds are simple, without secondary ones (Fig. 1E). Numerous well-developed tubular glands with secretory granules are present in the lamina-propia submucosa. A very scantly, connective tissue was observed interspace between the tubular glands (Fig. 1G and I). In the magnum of the aged nonlaying hen the mucosal epithelium is pseudostratified columnar with ciliated cells (Fig. 1H and J), and de mucosal folds are much less voluminous and tend to be complex with short secondary folds (Fig. 1F). In the lamina-propia submucosa, the gland tissue is markedly reduced, the tubular glands do not contain secretory products, and connective tissue is very abundant (Fig. 1H and J).

In mature and aged hens, the tunica muscularis, containing inner and outer longitudinal layers, was present, and the tunica serosa encloses the magnum (Fig. 1E and F).

Download English Version:

https://daneshyari.com/en/article/5506847

Download Persian Version:

https://daneshyari.com/article/5506847

Daneshyari.com