Influence of estradiol administration on estrogen receptors of nasal mucosa: an experimental study on guinea pigs

Henrique Olival Costa, Ney Penteado de Castro Neto, Lia Mara Rossi, Ieda Millas, Flavia Coelho, Leonardo da Silva*

Santa Casa de São Paulo, Faculdade de Medicina, São Paulo, SP, Brazil

Received 29 March 2013; accepted 12 October 2013

Abstract

Introduction: Some clinical trials revealed a correlation between increased serum estrogen and nasal symptoms or inflammatory changes in nasal mucosa. Estrogen receptors tend to be controlled by a negative feedback, to avoid a deleterious stimulus over several body functions while in hyperestrogenic periods. This study proposes a hypothesis where mechanisms regulating expression of estradiol receptors in nasal mucosa are absent in some patients, and their concentration remains steady even in periods of high serum hormonal concentration, potentially leading to local estrogenic symptoms in nasal mucosa.

Study design: This was an experimental prospective study.

Aim: To determine whether estrogen levels induce the reduction of the number of estrogen receptors in the nasal mucosa.

Methods: In the present study, 30 adult male guinea pigs were subjected to a biopsy of the middle nasal turbinate and received 0.5 mL of estradiol cypionate intraperitoneally for 30 consecutive days. Afterwards, samples from contralateral middle turbinate were obtained. Immunohistochemical analysis of estrogen receptors were performed pre- and post-treatment.

Results: The post-treatment group showed reduction of receptor expression when compared to the pre-treatment group. (p = 5.2726-5).

Conclusion: A reduction in the expression of the nasal estrogen receptor was observed after 30 days of estradiol administration.

© 2014 Associação Brasileira de Otorrinolaringologia e Cirurgia Cérvico-Facial. Published by Elsevier Editora Ltda. All rights reserved.
periodos de elevada concentração sérica hormonal, o que pode conduzir a sintomas locais na mucosa nasal.

**Desenho do estudo:** estudo prospectivo experimental.

**Objetivo:** Determinar se altos níveis de estrogênio induzem à redução no número de receptores de estrogênio na mucosa nasal.

**Material e método:** Trinta cobeias foram submetidas à biópsia da concha nasal, recebendo 0,5 ml de cipionato de estradiol por via intraperitoneal por trinta dias consecutivos. Em seguida foram obtidas amostras da concha nasal contralateral. As análises imunohistoquímicas dos receptores de estrógeno foram realizadas antes e depois da hormonioterapia.

**Resultados:** O grupo pós-tratamento mostrou uma redução da expressão dos receptores (p = 5,2726-5).

**Conclusão:** Redução na expressão do receptor de estrogênio nasal foi encontrada após trinta dias de administração de estradiol.

© 2014 Associação Brasileira de Otorrinolaringologia e Cirurgia Cérvico-Facial. Publicado por Elsevier Editora Ltda. Todos os direitos reservados.

**Introduction**

Rhinitis may be classified into two groups: allergic and non-allergic. The non-allergic group includes hormonal rhinitis, in which inflammatory alterations in nasal mucosa are associated to elevated hormone levels, such as hyperestrogen states occurring during pregnancy, menstruation, or with use of hormonal contraception.1-3

Hormonal rhinitis has been the focus of clinical trials since the 19th century, when several authors published studies correlating hormonal fluctuations with nasal symptoms. Many authors4-7 carried out experimental studies disclosing histological and histochemical changes in nasal mucosa in response to changes in the concentration of serum estrogen.

Some clinical trials8-16 observed a correlation between increased serum estrogen and nasal symptoms or inflammatory changes in nasal mucosa on clinical and laboratory exams. Conversely, other studies, such as those by Mabry,17 by Ellegard,18,19 by Bende and Gredmark,20 and by Salaroli et al.21, failed to confirm this correlation.

Toppozada et al.,22-24 observed histological and histochemical alterations in the human nasal mucosa of women who were pregnant or using contraceptive pills, compared with a control group. Caruso et al.12 and Nappi et al.25 noticed that the vaginal and nasal respiratory epithelium exhibited the same histologic aspects in the respective phases of the menstrual cycle and during menopause.

The first estrogen receptor (ER) to be described, alpha (ERα), was isolated by Elwood Jensen in 1958. In 1996, Enmark and Gustafsson26 discovered a second type of receptor, beta (ERβ). Theories proposed about the physiological functions of these two isoforms implicate selective action of estrogen in different tissues.

Thus, research into the two receptors subtypes has been conducted in a range of different organs and tissues. ERB was found in the prostate, ovaries, testicles, uterus, hypophysis, bladder, lungs, salivary glands, oral mucosa, thymus, adrenals, olfactory tract, central nervous system, heart, kidneys, and in cells of the immunologic system.27 ERα was found at highest concentrations in the uterus, vagina, and breasts;28 however, few studies investigating ERs in nasal mucosa are available.

The mucosa of lower nasal turbinates in women with chronic rhinopathy was investigated by immunohistochemistry for ERs, which were detected in the cytoplasm of glandular epithelium cells.29,30 Millas et al.31 employed immunohistochemistry to study estrogen ERα and ERβ in the mucosa of lower nasal turbinates of normal subjects. All 11 cases studied (five women and six men) presented ERα and ERβ (predominantly the latter) in the cytoplasm of glandular epithelial cells of the lamina propria. When studying individuals with chronic rhinopathy, Shirasaki et al.32 observed ERα in the nuclei of mastocytes, and ERβ in nuclei of cells of the glandular epithelium of the lamina propria.

Controversy remains over the site and function of receptors for estrogen in nasal mucosa. Millas et al.33 assessed the influence of oral contraceptives on the distribution and density of ERs in the nasal mucosa of women, and observed that those using contraceptive pills had a lower number of receptors in the lamina propria.

The expression of receptors in cells is dynamic and regulated mainly by the concentration of their cognate ligands. Reports by Jensen and Gorski describing the regulation of ER concentration observed that estrogen treatment led to a decrease in cytosolic ERs, based on the reduction in specific binding to 17β-estradiol.

Given that the receptors are the key determinants of the action of several hormones in target cells, and that evidence points to a possible estrogen activity in nasal mucosa, the mechanism of down-regulation may explain the fact that, in general, women do not present significant nasal effects when there is marked variation in their sexual hormones.

Inversely, a hypothesis may be proposed that mechanisms regulating receptors expression are absent and that their concentration remains steady even during high hormonal concentration, potentially leading to local estrogenic symptoms in nasal mucosa. This study aimed to determine whether an elevation in circulating estrogen levels influences the concentration of ERs in guinea pig nasal mucosa.

**Materials and methods**

A prospective study of nasal mucosa samples from 30 adult male guinea pigs weighting between 300 g and 400 g was performed.
The procedures were conducted in accordance with the regulations of the research ethics committee of the Unit for Experimental Surgery Technique and complied with Brazilian Federal Law No. 1135/95 as well as with the ethical principles for animal experiments established by the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal - COBEA).

**Procedures**

The surgical procedures were performed under anesthesia with tiletamine chlorohydrate 125 mg/5 mL and zolazepam chlorohydrate 125 mg/5 mL, at a dose of 0.4 mL/kg, in addition to fentanyl citrate + droperidol. Guinea pigs were submitted to a biopsy of left nasal mucosa material using a punch biopsy forceps, obtaining fragments measuring approximately 2 × 2 × 2 mm. All fragments were immersed immediately upon collection in 4% buffered formalin and sent for histologic processing.

Guinea pigs were given 0.5mL of estradiol cypionate, sterile solution, 2mg/mL (E.C.P.™, Pfizer) intraperitoneally, daily for 30 days. The guinea pigs were given access to feed and water *ad libitum*. After 30 days of hormonal therapy, the guinea pigs were sacrificed by injection of a lethal dose of potassium chloride under anesthesia, and submitted to exposure and removal of the nasal mucosa of the middle turbinate contralateral to the initial biopsy site.

**Immunohistochemical analysis**

The tissue samples underwent sequential immersion in buffered formalin for 1h, immersion in absolute alcohol (six times in different recipients, each for 1h), immersion in xylol (four times in different recipients, each for 1h), and immersion in liquid paraffin (twice in different recipients, each for 1h), and immersion in liquid paraffin (twice in different recipients, each for 1h). Subsequently, specimens were embedded in liquid paraffin. The paraffin blocks for ent recipients, each for 1 h). Subsequently, specimens—1h), and immersion in liquid paraffin (twice in different recipients, each for 1h). Subsequently, specimens were immersed in xylol (four times in different recipients, each for 1h), and immersion in absolute alcohol (six times in different recipients, each for 1h), and immersion in liquid paraffin (twice in different recipients, each for 1h). Specimens were immersed in 4µm-thick slices and mounted on electrically charged slides, with each slide holding two slices. The slides were sent to the pathology laboratory of the Brigham and Women’s Hospital, Boston, United States, where blocks were cut into 4µm-thick slices and mounted on electrically charged slides, with each slide holding two slices. The slides were then sent to the pathology laboratory of the Brigham and Women’s Hospital, Boston, United States, for immunohistochemical processing. In this procedure, the slides were submitted to a deparaffinization process by immersion for 10 minutes in Hemo-De™ (Scientific Safety Solvents - Texas, USA), followed by immersion in six different recipients of absolute alcohol. Endogenous peroxidase blocking was then performed by immersion in a solution constituting 30% volume of 3% hydrogen peroxide and 70% volume of absolute alcohol. The slides were then washed in running water for 3 minutes.

Antigenic recovery was then performed by immersion in buffered pH 6-citrate solution pre-heated to 25°C for 30 minutes. The sections were delimited using a pap pen™ (SciTek Laboratories). Slides were left for 1 minute in a recipient containing 500mL of PBS with 10 mL of 1% BRU. After removal from this solution, the non-specific primary anti-body H222 was applied (marker of both ERα and ERβ) for ERs at a final concentration of 5µg/mL, and submitted to overnight incubation at 4°C in a wet chamber. Following incubation, slices were rinsed with PBS to remove excess primary antibody and then re-immersed in PBS/BRU 1% solution for 10 minutes. Slices were incubated with the secondary antibody Mouse Envision+™ (DakoCytomation - Glostrup, Denmark) for 30 minutes, rinsed again with PBS, and immersed in a PBS/BRU 1% solution for 10 minutes. Sections were then incubated in a solution of DAB+™ (DakoCytomation), 1 drop per mL of buffer for 10 minutes. Upon removal from incubation, slides were rinsed in running water, counterstained with Mayer’s hematoxylin (1.5 minutes), rinsed again, submitted to Scott’s water (1 minute), and again washed in running water. Sections were then dehydrated, dried, and mounted with coverslips.

Slides were analyzed pairwise, comparing the images obtained before and after hormone therapy through relative optical density (ROD™) analysis.

ROD is a software for quantitative imaging assessment that estimates the quantity of solid aggregates in a single bi-dimensional image, after due calibration for a known vertical thickness (immunohistochemistry slide) (Fig.1). The methodology reduces the subjectivity of the observations and enables numerical determination of the tissue stained, facilitating comparison between pairs. For each specimen, four separate pre- and post-therapy slides were assessed, and the average result was used.

**Results**

Results were compared using the parametric Student’s *t*-test for paired samples (pre- and post-hormone therapy).

The results of the paired group analysis are shown in Table 1.

Animals 16 and 19 presented no glands in their respective histological slices, whereas the post-therapy slides for animal 23 were lost.

![Image](image-url)
**Table 1** Optical density of slides before and after estradiol treatment.

<table>
<thead>
<tr>
<th>Animals</th>
<th>ROD</th>
<th>Animals</th>
<th>ROD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.14625</td>
<td>1</td>
<td>0.12415</td>
</tr>
<tr>
<td>2</td>
<td>0.109225</td>
<td>2</td>
<td>0.133775</td>
</tr>
<tr>
<td>3</td>
<td>0.102725</td>
<td>3</td>
<td>0.098295</td>
</tr>
<tr>
<td>4</td>
<td>0.176725</td>
<td>4</td>
<td>0.09625</td>
</tr>
<tr>
<td>5</td>
<td>0.144075</td>
<td>5</td>
<td>0.114875</td>
</tr>
<tr>
<td>6</td>
<td>0.14935</td>
<td>6</td>
<td>0.119275</td>
</tr>
<tr>
<td>7</td>
<td>0.1679</td>
<td>7</td>
<td>0.132025</td>
</tr>
<tr>
<td>8</td>
<td>0.1308</td>
<td>8</td>
<td>0.10255</td>
</tr>
<tr>
<td>9</td>
<td>0.137575</td>
<td>9</td>
<td>0.126675</td>
</tr>
<tr>
<td>10</td>
<td>0.1528</td>
<td>10</td>
<td>0.0935</td>
</tr>
<tr>
<td>11</td>
<td>0.17205</td>
<td>11</td>
<td>0.11185</td>
</tr>
<tr>
<td>12</td>
<td>0.146625</td>
<td>12</td>
<td>0.12415</td>
</tr>
<tr>
<td>13</td>
<td>0.109225</td>
<td>13</td>
<td>0.133775</td>
</tr>
<tr>
<td>14</td>
<td>0.102725</td>
<td>14</td>
<td>0.098295</td>
</tr>
<tr>
<td>15</td>
<td>0.176275</td>
<td>15</td>
<td>0.09625</td>
</tr>
<tr>
<td>17</td>
<td>0.144075</td>
<td>17</td>
<td>0.114875</td>
</tr>
<tr>
<td>18</td>
<td>0.14935</td>
<td>18</td>
<td>0.119275</td>
</tr>
<tr>
<td>20</td>
<td>0.1679</td>
<td>20</td>
<td>0.132025</td>
</tr>
<tr>
<td>21</td>
<td>0.1308</td>
<td>21</td>
<td>0.10255</td>
</tr>
<tr>
<td>22</td>
<td>0.137575</td>
<td>22</td>
<td>0.126675</td>
</tr>
<tr>
<td>24</td>
<td>0.1528</td>
<td>24</td>
<td>0.0935</td>
</tr>
<tr>
<td>25</td>
<td>0.17205</td>
<td>25</td>
<td>0.11185</td>
</tr>
</tbody>
</table>

**Statistical analysis**

The parametric Student’s *t*-test was performed using the ROD’s results of samples from 19 animals from pre- and post-therapy slides. The groups were statistically different; the pre-treatment group presented a higher number of receptors than the post-treatment group (*p* = 0.000005726).

**Discussion**

Based on clinical observations, a number of studies indicate a correlation between hormonal changes and nasal obstruction. Hormonal rhinitis is cited in various otorhinolaryngology textbooks, yet its underlying physiopathological mechanism remains unclear. Many studies have already indicated the activity of feminine sexual hormones over the nasal mucosa. It is postulated that, in situations of increased serum hormone (estrogen) levels, there is a stimulus for increased expression of its receptors, triggering changes in the density and localization of these receptors.

In the present study, the influence of estradiol administration on the expression of ERs in the nasal mucosa of guinea pigs was observed.

Some issues were observed during the course of the present study, including excessive tissue fragmentation in the pre-estrogen treatment group, which was most likely due to the tissue collection technique employed. The specimens were taken with a small punch biopsy forceps introduced into the nasal cavity. Consequently, the exact site of tissue collection was not precise; besides, the mechanism of action of the instrument (gripping and pulling) may also have led to excessive tissue laceration, evidenced on the
the ER concentration of the nasal mucosa of post-menopausal women. The authors declare no conflicts of interest.
Influence of estradiol administration on estrogen receptors of nasal mucosa: an experimental study on guinea pigs