Pregnancy rhinitis: pathophysiological effects of oestrogen and treatment with oral decongestants

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ABSTRACT

Pregnancy rhinitis, a common condition, is thought to affect about 20% of pregnant women. This type of rhinitis may develop at any time during pregnancy and the nasal stuffiness usually disappears shortly after delivery. This condition is troublesome and can interfere with sleep, induce tiredness and dryness of the mouth. It is difficult to treat and no specific treatment seems to be entirely satisfactory. The aetiology of pregnancy rhinitis and the effect of oestrogen on the nasal mucosa remain to be clarified.

For purposes of research, it is essential to evaluate subjective complaints of nasal obstruction. Several methods can be used to rate sensory intensities. The "telephone scoring system" combined with a numerical rating scale (NRS) uses a computer to check the time when the estimate is made. Comparison of the symptoms estimated with two methods, a visual analogue scale (VAS) and “telephone scoring system” combined with a NRS show a strong correlation and symptom scores are similar. However, the patients find the latter system easier to use.

Phenylpropanolamine (PPA), a nasal decongestant given orally in sustained-release preparations, is commonly prescribed to relieve nasal blockage in viral infections of the upper airways and in allergic rhinitis. PPA in double the recommended dose has an excellent decongestive effect on the nasal mucosa of healthy subjects, and it has the same decongestive effect as oxymetazoline nasal spray, but the systolic and diastolic blood pressures also increase temporarily. When PPA is given in the recommended dose, as assessed by the “telephone scoring system”, it relieves nasal stuffiness in women with pregnancy rhinitis and has no effect on blood pressure.

The increase in oestrogen levels in blood during in vitro fertilization (IVF) in a group of healthy women causes hyperreactivity of the nasal mucosa after histamine challenge. However, no increase in nasal mucosal swelling is noted with low and high oestrogen levels before histamine challenge although perfusion and velocity tend to increase at high oestrogen levels as measured with laser Doppler flowmetry. The increase in perfusion and velocity after histamine challenge is lower at high than low oestrogen levels.

In the human nasal mucosa of healthy subjects, immunocytochemistry showed distinct oestrogen receptor (ER) β positive cells in all sections. These cells, located subepithelially and close to the basal membrane, had no relation to the vascular or glandular structures or to the epithelium. They were comparatively large with a centrally-located nucleus. Immunoreactive signals for ER β proteins were seen in their nuclei. Weak signals of ER β immunostaining in the cytoplasm were also detected, which may indicate the presence of ER β proteins in the cytoplasm. However, subglandular, endothelial and epithelial cells showed no immunoreactivity for ER β in the nasal mucosa. Double immunostaining showed co-expression of ER β with the specific mast cell marker, anti-human mast cell tryptase, in the vast majority of ER β positive cells.

In conclusion, PPA in double the recommended dose has an excellent decongestive effect on the nasal mucosa of healthy subjects, and it has the same decongestive effect as oxymetazoline nasal spray. PPA given in the recommended dose relieves nasal stuffiness in women with pregnancy rhinitis and has no effect on blood pressure. These data indicate that PPA may be useful in the treatment of pregnancy rhinitis. In the human nasal mucosa of healthy subjects, we found ER β positive cells. The increase in oestrogen levels in blood during IVF in a group of healthy women causes hyperreactivity of the nasal mucosa after histamine challenge. These findings indicate that oestrogen may play a role in the development of pregnancy rhinitis.

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Paper IV Toll K, Graf P, Backheden M, Stjärne P. Evidence that nasal mucosal hyperreactivity in healthy women is induced by high levels of estrogen. Submitted for publication

LIST OF ABBREVIATIONS

ENT = Ear, nose and throat
ER = Oestrogen receptor
FSH = Follicle-stimulating hormone
GrH = Gonadotropin-releasing hormone
HE = High concentration of s-estrogen
IVF = In vitro fertilisation
LDF = Laser-Doppler flowmetry
LE = Low concentration of s-estrogen
NARES = Persistent non-allergic rhinitis with or without eosinophilia
NRS = Numerical rating scale
PGH = Placental growth hormone
PPA = Phenylpropanolamine
PNEF = Nasal expiratory peak flow
PNIF = Nasal inspiratory peak flow
s-estrogen = Concentration of estrogen in blood serum
VAS = Visual analogue scale
VIP = Vasoactive intestinal polypeptide
Introduction

Rhinitis affects nearly 25% of the world’s population. It is not a single disease but a heterogeneous group of disorders with diverse pathophysiological mechanisms that are not always inflammatory. The symptoms consist of one or more of the following: rhinorrhea, sneezing, itching and/or nasal obstruction as well as a reduced sense of smell. They may have more than one cause – e.g., anatomic obstruction, infection, underlying systemic disease, allergy or non-allergic inflammation. Although the symptoms of allergic and non-allergic rhinitis may be similar, these subtypes can be distinguished clinically. Examples of non-allergic causes of rhinitis include infectious rhinitis, rhinitis medicamentosa, persistent non-allergic rhinitis with or without eosinophilia (NARES), atrophic rhinitis, drug-induced rhinitis and rhinitis caused by conditions involving hormones. Hormonal rhinitis is frequently associated with hypothyroidism, acromegaly and pregnancy, but it has also been noted in postmenopausal women and older men. Although the precise mechanisms underlying hormonal rhinitis are disputed, some studies of sex hormones suggest that these may cause nasal inflammation.

Far fewer recommendations concerning specific diagnostic criteria and treatment for non-allergic rhinitis are available than those for allergic rhinitis.

The diagnosis of rhinitis begins with a careful history and physical examination. As regards the history, one should focus on the characteristics of the patient’s symptoms, their frequency, and severity. The examination should include general inspection of the ears, nose and throat, together with rigid or flexible nasoendoscopy.

Both allergic and non-allergic rhinitis are important risk factors for asthma and bronchial hyperreactivity. The pathophysiological processes that occur in the nasal mucosa with rhinitis and in the lower airways with asthma are believed to reflect common manifestations of a generalized inflammatory respiratory disorder. The presence of rhinitis can predict the degree of bronchial hyperreactivity, even in patients who have not yet been diagnosed as having asthma.

The disease is sometimes ignored because it is not life-threatening, but it seriously affects the patient’s quality of life, productivity and health care costs. One of the commonest complaints caused by rhinitis is disturbance of sleep. Poor-quality sleep leads to daytime drowsiness, fatigue, indecision, and significant impairments in learning.

Pregnancy rhinitis

Pregnancy rhinitis, a common condition, is thought to affect 9-42% of pregnant women. This type of rhinitis may develop at any time during pregnancy and the nasal stuffiness usually disappears shortly after delivery. Some defined pregnancy rhinitis: “as nasal congestion in the last 6 or more weeks of pregnancy without other signs of respiratory tract infection and with no known allergic cause that disappear completely within 2 weeks after delivery.” With this definition, the incidence is 22%. The condition is troublesome and can interfere with sleep, induce tiredness and dryness of the mouth. Comparisons of health-related quality of life scores in patients with pregnancy rhinitis or other types of rhinitis and healthy controls show significant differences in these parameters.
The possible effects on the foetus have also been discussed. Difficulty in breathing through the
nose increases mouth breathing and snoring. Snorers have higher frequencies of hypertension,
pre eclampsia and of infants with intrauterine growth retardation 16,17.

The aetiology of pregnancy rhinitis remains to be clarified. Many authors have studied the
relationship between hormones and pregnancy rhinitis and the effects of numerous hormones and
substances. For example, progesterone, which has a vasodilating effect, was thought to cause
pregnancy rhinitis but some studies have shown that the serum levels of progesterone are similar
in women with or without this condition 18.

Vasoactive intestinal polypeptide (VIP) and prolactin have also been implicated, but recent
studies do not support these theories 19.

One author reported that serum levels of placental growth hormone (PGH) were significantly
higher in women with pregnancy rhinitis. However, that study included only five women with
pregnancy rhinitis 18.

Oestrogen as a cause of nasal stuffiness is supported by the observation that patients with
atrophic rhinitis who are treated with topical oestrogen develop congestion of the nasal mucosa 4.
It has also been noted that contraceptive pills with high levels of oestrogen can induce congestion
of the nasal mucosa as a side-effect 20. Ultrastructural and histochemical examinations show
squamous metaplasia of the nasal mucosa in these patients 21. Skin test reactivity in the menstrual
cycle indicates that the wheal-flare reaction is significantly greater in the mid-cycle when
oestrogen levels reach their peak 22. Moreover, the nasal mucosa becomes hyperreactive to
histamine during ovulation, when the blood levels of oestrogen peak, which may explain why
nasal stuffiness occurs in pregnancy 23.

Treatment of pregnancy rhinitis

Pregnancy rhinitis is difficult to treat and no specific treatment seems to be entirely satisfactory.
Topical decongestants relieve the symptoms temporarily, but regular over-use of topical
decongestants may result in nasal hyperreactivity and hypertrophy of the nasal mucosa, leading to
a condition called rhinitis medicamentosa 24,25. The influence of topical decongestants on
the foetus has also been discussed 26. Topical steroids are effective in the treatment of many kinds of
rhinitis, such as allergic rhinitis, persistent non-allergic rhinitis, rhinitis medicamentosa and nasal
polyps 27,28,29,30,31. Although they are also widely used to treat pregnancy rhinitis, the only
controlled clinical study showed no effect of nasal steroids compared with placebo 32. This would
suggest that pregnancy rhinitis is not due to an inflammation and this should be studied further.
Systemically-administered steroids have been given to patients with various types of rhinitis, but
no clinical data are available on the safety or efficacy of these treatments during pregnancy 33,34.

Methods of measuring changes in the human nasal mucosa

To understand the pathogenesis of nasal mucosal inflammation and distinguish normal mucosal
reactions from pathological, one objective methods must be used to measure the state of the
mucosa 35. These should be non-invasive to minimize artefacts, and suitable for the human nasal
mucosa. However, some methods are more appropriate for certain purposes 36,37.
Nasal mucosal congestion can be assessed directly or indirectly. The commonest indirect methods are rhinomanometry and nasal inspiratory and expiratory peak flow (PNIF and PNEF). They measure the effects of congestion of the nasal mucosa on nasal airway flow and resistance. Rhinomanometry is the most widely used method for indirect measurement of nasal mucosal congestion. A close anterior rhinomanometry, the most common rhinomanometric method, records nasal airflow and air-pressure differences during quiet breathing in one cavity at a time. It permits an objective evaluation of surgery on cartilage and bone if the mucosa is decongested. It is also of value in the selection of patients who, after functional septoplasty, can expect a good surgical result, but less satisfactory for measurements of nasal hyperreactivity.

Both PNIF and PNEF are inexpensive, quick and easy for patients to use, can be done repeatedly and the findings compare well with rhinomanometry. In most instances the nasal inspiratory peak flow technique is better than the expiratory peak flow one, because the PNEF measurements are affected by mucus in the flow meter. They are useful in clinical studies in which the patients can check their airflow at home.

Another indirect method, acoustic rhinometry, measures the cross-sectional areas at various distances from the nostril. Rhinostereometry is a direct method in which the nasal cavity is viewed through an operating microscope. The latter two methods are described on page 14 and 17.

An important methodological difference, as regards rhinomanometry, acoustic rhinometry and rhinostereometry, is that in the latter, the external nose is held open by a self-retaining nasal speculum and the anterior part of the nasal cavity is viewed under magnification by means of an operating microscope. Therefore measuring instruments, such as probes in the nose, can be introduced and their position monitored under direct visual control and without touching the nasal mucosa.

Symptom scoring

Many studies are based on symptom scores. The most popular method is one in which the patients record their symptoms in a diary at a certain time. Several methods can be used to rate sensory intensities. The visual analogue scale (VAS) is frequently used in clinical works. Usually it consists of a 10 cm line on a paper which is shown to the patients, who are asked to indicate the severity of their symptoms by putting a mark along the line with a pencil. Another method for assessing symptoms is to use a numerical rating scale (NRS) with several many numbers. With both methods the patients are usually asked to fill in a diary at certain times, for example, in the morning and evening. However, it is well-known that some patients forget to do this and they make entries in the whole diary on the last day of the trial.

A third method, the telephone scoring system, uses a computer to check the time when the estimates are made. The patients phone the computer and receive stepwise instructions. In our study we used a NRS with ten alternatives (0-9) to estimate the severity of symptoms. The results and the times of the call were registered by the computer. The great advantage of the method is that the computer records the time when the estimate is made. In this study, we wanted the patients to score their symptoms in the morning and evening. Some of them went to bed in the middle of the night and woke up in the middle of the day. With this method, we could see these variations afterwards and exclude them.
Phenylpropanolamine

Phenylpropanolamine (PPA), a widely used nasal decongestant given orally in sustained-release preparations, is commonly prescribed to relieve nasal blockage in viral infections of the upper airways and in allergic rhinitis 48,49,50. PPA, which is known in Sweden as Rinexin® (Recip, www.recip.se) has been available on the market for more than 50 years. In many countries, it is available over-the-counter, but in Sweden a prescription is necessary and the recommended dose is 50 mg twice daily.

Unfortunately, only a few controlled clinical studies have been done using objective measurements to assess its decongestive effects.

The action of PPA is not understood, but it may be mediated directly by activation of post-junctional adrenoreceptors, indirectly by affecting the release and/or re-uptake of noradrenalin or by both mechanisms51.

In the USA, PPA has been withdrawn from the market because of a few cases of cerebral haemorrhage 52. However, those patients took more than the recommended dose to lose weight. Some clinical studies show that when the recommended dose is exceeded, the blood pressure increases but if the recommended dose is used, PPA has no clinically significant cardiovascular effects 53,54.

Hardly any data are available that suggest a negative effect of PPA on foetal development. An association between the maternal use of PPA and gastroschisis or small-bowel atresia has been discussed in retrospective case-control studies of maternal drug exposure and congenital malformations. A relation between the use of PPA and gastroschisis or small-bowel atresia was found in one study, but not in another 55,56,57. In a recent large study no teratogenic effect of oral decongestants was found. They evaluated delivery outcome and included the presence of congenital malformations after maternal use of oral decongestants during pregnancy, as well as information about drug use that had been collected before pregnancy outcome was known 58.

Oestrogen receptors

Oestrogens are steroid hormones that have profound effects on both the male and female reproductive systems. They also play important roles in the cardiovascular system and in the maintenance of bone tissue. These effects are all mediated by oestrogen receptors (ER) 59,60. The first ER was cloned in 1986. This receptor was regarded as the only ER until a second ER was reported ten years later 61. Today they are known as ER-alfa and ER ß. Both receptors are very similar at the amino acid level but their effects when activating these receptors differ greatly. ER-alfa and ER ß can be detected in many tissues. In some organs, both receptor subtypes are expressed at about the same level, but in others, one or the other subtype predominates. Moreover, both receptor subtypes may be present in the same tissue, but in different cell types. For example, ER-alfa is mainly expressed in the uterus, prostate, ovary, testes, bone, breast, liver and white adipose tissue while ER-ß is expressed in the colon, lungs, brain, vascular endothelium and salivary glands 62.

The role of oestrogen in the airways is still not fully understood and the findings are disputed 63. In the lungs, ER ß is highly expressed in pneumocytes and bronchiolar epithelial cells. It plays an
important role in the development of the lungs and its loss leads to abnormal lung structure and systemic hypoxia in mice \(^{64,65}\). Only a few studies have assessed the expression of ER-alfa and ER ß in the upper airways and their function remains to be clarified \(^{66,67}\).

**Mast cells**

Mast cells are key effector cells in a variety of allergic disorders and chronic inflammatory diseases including nasal polyposis and asthma \(^{68,69,70}\). It is well known that degranulation of mast cells leads to both microvascular and cellular responses, causing contractions and rapid oedema of smooth muscles and mobilising other inflammatory cells to enter the inflammatory site. The more recent discovery that mast cells are sources of cytokines which play key roles in signal transduction and communication between immune cells suggests that mast cell activation may affect the development of T and B cell responses \(^{71}\). Mast cells in nasal polyps express oestrogen receptors and progesterone receptors \(^{67}\). These findings indicate that mast cells may be the primary target responsible for the effects of sex hormones in the airways.

**Aims of the present study**

1. To compare the onset and duration of the decongestive effects of 50 and 100 mg phenylpropanolamine (PPA) in healthy subjects for 8 hours, using rhinostereometry and acoustic rhinometry. An additional aim was to study the possible effects on the systolic and diastolic blood pressures.

2. To evaluate a new method of scoring symptoms with which we could check the time when estimates were made and to compare this method with use of a visual analogue scale (VAS) and a diary.

3. To evaluate the decongestive effect of phenylpropanolamine (PPA) 50 mg twice daily for seven days in women with pregnancy rhinitis by using rhinostereometry and the subjective assessment of symptoms of nasal stuffiness. An additional aim was to study the possible effects of PPA on the systolic and diastolic blood pressures.

4. To determine whether changes in oestrogen levels in the blood affect nasal mucosal reactivity and the microcirculation when the concentrations of other hormones remain constant.

5. To investigate the expression and location of oestrogen receptor ß (ER ß) in the human nasal mucosa.
Subjects and methods

Study populations
In Paper I, we studied 15 healthy volunteers (7 women, 8 men) who were students at the University of Stockholm. They were all clinically healthy and had no history of allergy or other rhinological disease.

In Paper II, we assessed 60 patients with allergic rhinitis, all of whom were allergic to birch. 30 were from Ullevål University Hospital, Oslo, Norway and 30 from Soder Hospital, Stockholm, Sweden.

In Paper III, we evaluated 40 pregnant women with pregnancy rhinitis who had complained of persistent nasal obstruction for at least two weeks and were patients at Munkbron’s Antenatal Clinic in Stockholm. Apart from pregnancy rhinitis, they were in good health and had no history of allergy or other rhinological disease, high blood pressure or toxaemia in a previous pregnancy.

In Paper IV, we studied 15 healthy women who were undergoing in vitro fertilization (IVF) for treatment of unexplained infertility. They had no history of allergy or other rhinological disease.

In Paper V, we examined the nasal mucosa of the medial turbinate from 8 patients (4 women, 4 men; age 21-43) who underwent nasal septal surgery. They were all healthy patients and had no history of allergy or other rhinological disease.

All the studies were approved by the local ethics committee.

Rhinostereometry

This very accurate optical, direct, non-invasive method was designed to measure nasal mucosal swelling. Topographic measurements can therefore be made in the nose, without manipulation of the nasal structures. In rhinostereometry, the origin of a three-dimensional coordinate system is used as the fixed point. The apparatus consists of a surgical microscope placed on a micrometer table fixed to a frame. The microscope can be moved in three angular directions, thus establishing the coordinate system. To permit measurements, the nasal cavity must be placed in the coordinate system in such a way that it resumes the same position with a high degree of precision during repeated measurements. This is achieved by immobilising the subject’s head exactly to the frame by an individually-formed tooth splint 72,73.

The nasal cavity is viewed through the eyepiece of the microscope. Since the microscope has a small depth of focus, a sharply delineated area of the surface is examined in the plane focus. As the ocular is equipped with a horizontal mm scale, the sharply delineated area, vertically directed, crosses the mm scale and its position can be recorded. With this method, the area of measurement can be re-identified with a high degree of accuracy in the same individual at various times with the subject as his own control 74,75.

Rhinostereometry permits measurements of all visible parts of the nasal mucosa. However, in this study, we chose the mucosal surface of the medial side of the inferior concha. This region is part of the valve area, which is considered to play a major role in the pathogenesis of nasal obstruction. Moreover, it consists of erectile tissue with high reactivity. These measurements were made in Papers I, III and IV. In Paper I, we also used acoustic rhinometry because rhinostereometry was not well known then. Today, rhinostereometry has been used in several studies 76,77,78.
Laser-Doppler flowmetry

Laser-Doppler flowmetry (LDF), the only non-invasive method for studying the microcirculation, permits continuous and instantaneous measurements of nasal mucosal blood flow. Non-invasiveness is of great importance because it avoids artefacts. Several multiple microcirculatory parameters, can be determined - i.e., the concentration of moving blood cells (CMBC), the velocity of the blood flow (V) and the product of these two, perfusion or flow (P)\(^7\). Some authors have used laser-Doppler recordings from the nasal mucosa to make quantitative measurements - i.e., in mL/100 g tissue/min\(^4\).

Laser-Doppler flowmetry in combination with rhinostereometry

Since no comparisons with other methods that measure blood flow in specific tissue are available, we express the data in arbitrary units, perfusion units (PU), concentration units (CU), and velocity units (VU). In our study we used a Periflux 4001 (Perimed, Sweden). The wavelength of the laser beam was 780 nm. We also used specially-designed probe and a micro-manipulator to position the probe tip under microscopic visual control at a well-defined distance from the mucosa\(^8\). The measuring distance should be 0.3 mm. At this distance it is easy to avoid touching the mucosa with the probe. LDF measures blood flow in the superficial parts of the nasal mucosa. In Paper IV, the nasal mucosa was studied with rhinostereometry and laser-Doppler flowmetry during challenge with histamine. The combination of these methods has many advantages. This combination permits the use of two non-invasive methods for direct and
simultaneous measurements of congestion and the microcirculation because rhinostereometry allows the laser probe to be in place in the nose during the measurements of congestion. With rhinostereometry, one can study changes in the position of a small area of the nasal mucosal surface during various conditions. The degree of congestion can be compared in the same subjects at different times. However, rhinostereometry alone shows only the position of the surface, and the degree of congestion. It gives no information about the conditions of the mucosa. Since the inflammatory reactions in the nose consist mainly of changes in the vascular state in different layers of vessels, it seems wise to combine the assessment of congestion with a method for measuring changes in microcirculation. Laser-Doppler flowmetry seems to be the best method because like rhinostereometry, it is a non-invasive optical method that permits continuous recordings of microcirculatory parameters.

Laser Doppler flowmetry; probe in its position in the rhinostereometer.
Acoustic rhinometry

Acoustic rhinometry, a type of ultrasound, produces an acoustic pulse via a nasal adaptor that closely fits the nostril. It measures changes in congestion of the mucosa and records skeletal abnormalities. The cross-sectional area of the nasal cavity reflects to the distance into the nasal cavity. Therefore, acoustic rhinometry provides a two-dimensional view of the nasal cavity. This method is based on analysis of the amplitudes (i.e., the area) of sound waves, as reflections by the nasal cavity of an incident sound wave, the time taken by these waves to pass through the nasal cavity (i.e., the distance into the nasal cavity). In a non-decongested nose, three deflections or minimum notches are seen on the area-distance curve. The first notch may, in fact, be artefactual and the third notch is less clear, probably it corresponds to the head of the middle turbinate. The second notch is produced by the narrowest part of the nasal cavity, which is usually located no more than 3 cm from the nares. This minimal cross-sectional area, (MCA₂), is the area between the anterior portion of the concha inferior and the septum in the nasal valve area.
Telephone method

The severity of the symptoms can be assessed with the telephone scoring system, which records the time when the estimates are made with a computer. The patients phone the computer and receive stepwise instructions. In our study, we used a numerical rating scale (NRS) with ten alternatives (0-9) to estimate the severity of symptoms. The results and the times of the calls are registered by the computer. The great advantage of this method is that the computer records the time when the estimate is made. In Paper II, the patients were asked to score their symptoms in the morning and evening. Some of them went to bed in the middle of the night and woke up in the middle of the day. These variations could therefore be excluded afterwards 47.

In vitro fertilization

This part describes only the first part of in vitro fertilization (IVF) -i.e., ovarian stimulation, used in Paper IV.

Various methods are used for IVF, but at Karolinska University Hospital it is common to control the development of eggs, and start IVF when the concentrations of oestrogens in blood have been reduced to about zero. A nasal spray containing gonadotropin-releasing hormone, GrH (Suprecur®, Aventis Pharma A B; www.aventispharma.se) with benzalkonium chloride 0.1 mg/g as preservative, 1 puff (0.15mg) is sprayed into each nostril three times daily for about two to three weeks until the concentration of oestrogens in blood is < 200 pmol/l. This is followed by stimulation with subcutaneous injections of follicle-stimulating hormone, FSH (GONAL-f®, Serono Nordic A B; www.serono.com), 1 injection (150-300 E) daily for about 12-14 days. The levels of oestrogens in blood increase, which stimulate the ovaries and the development of eggs. To reduce the adverse effects such as the ovarian hyperstimulation syndrome, the patients continue to take GrH nasal spray, 1 puff daily (alternating between the right and left nostril). The development of eggs is followed with ultrasound 86.

Histamine challenge

Nasal challenge has been used to study physiological and pathophysiological mechanisms in the nasal mucosa. Histamine affects irritant receptors, and thereby stimulates the nerves and vessels which in turn cause mucosal swelling. Histamine was dissolved in 0.5 % phenol and NaCl and 0.14 ml of the solution was applied to the nasal mucosa in a concentration of 2.0 mg/ml. Previous studies have shown that provocation with this concentration seemed to be the critical dose. A syringe was used to place this solution on the mucosal surface of the medial side of the inferior concha on one side of the nose during visual inspection 87,88,89. The histamine challenge is used in Paper IV.

Immunocytochemistry

In Paper V biopsies were taken during nasal septal surgery from the inferior turbinate in one of the nasal cavities. The biopsied tissue was fixed using 4% paraformaldehyde in phosphate buffered saline (PBS). After fixation (1 hour), it was placed in 0.5% paraformaldehyde in PBS at 4°C, pending use. The tissue was then rinsed in PBS and placed in 20% sucrose for 48 h for
cryoprotection. The specimens were embedded in cryomount, quickly frozen in isopentane with dry ice, and stored at -20°C. Serial sections (10 \( \mu \text{m} \) thickness) were cut on a cryostat (HM 500M, Zeiss, Germany) at -24°C.

The sections were taken from the freezer, allowed to dry and subsequently washed with TBS for 5 min. Endogenous peroxidase was removed by using 3 % \( \text{H}_2\text{O}_2 \) in TBS for 10 min.

After rinsing with TBS three times, they were incubated with background sniper for 10 min to reduce the non-specific background (Biocare Medical BS966G, LLC, 4040 Pike Lane, Concord, CA, USA) and then rinsed again three times with TBS.

The antibodies used were: monoclonal mouse anti-human mast cell tryptase (DAKO Cytomation, Denmark, M 7052 Clone AA1) 0.1ug/ml and polyclonal rabbit oestrogen receptor ß (catalogue no. ab 3577, Abcam, Cambridge, UK) 5 ug/ml. 60 min RT.

After rinsing with TBS, the slides were incubated with mouse probe (Biocare Medical MP530G). They were rinsed again with rabbit probe (Biocare Medical RP531G). To visualize the monoclonal antibody mouse anti-human mast cell tryptase, the slides were incubated with alkalinephosphatase mouse-polymer (Biocare Medical MAP532G). The alkalinephosphatase was developed with the Vulcan fast Red Chromogen Kit (Biocare Medical FR804). To visualize the polyclonal oestrogen receptor ß antibody, the slides were incubated with horseradish rabbit-polymer (Biocare Medical RH531G). The HRP was developed with DAB+Ni (Vector SK4100). The slides, counterstained with Mayer HTX, were mounted with crystal mount (Biomeda) and then with DPX mounting (VWR England).

Monoclonal mouse anti-human mast cells tryptase from Dako Cytomation, Denmark, code no. M 7052 Clone AA1. Biocare Medical (www.biocare.net), Biocare Medical LLC, 4040 Pike Lane, Concord, CA 94520, USA. Vector (www.vectorlabs.com), Vector Laboratories Inc., 30 Ingold Road, Burlingame, CA 9410, USA. Biomeda, Novakemi (www.novakemi.se), VWR International (www.vwr.com).

The specificity of the antibody was tested by pre-incubation of the antibody with ER ß blocking peptide (catalogue no. ab3564, Abcam, Cambridge, UK) applied to sections, and as a negative control we used sections from mutant mice lacking the ER ß (BERKO mice). We also tested the effect of various fixatives on the staining pattern of ER ß. These fixatives (4% paraformaldehyde/1% acetic acid or 4% paraformaldehyde/1% acetic acid/0.1% glutaraldehyde) in PBS were compared to our standard fixation with 4% paraformaldehyde in PBS. The specificity of the antibody was further confirmed by Western blot with positive controls (data not shown) (ER ß 530, Panvera, Madison, WI, USA).
Results

Paper I
Effects of sustained-release oral phenylpropanolamine on the nasal mucosa of healthy subjects

Rhinostereometry showed that phenylpropanolamine (PPA) 100 mg had a significant decongestive effect and reached its maximal effect after 2 h. The effect lasted for 5 h and then it gradually declined. The mean decongestive effect of 100 mg PPA between 2 h and 6 h was significantly greater than the corresponding effect of 50 mg PPA and placebo. We found no significant difference between 50 mg and placebo during the 8-h observation period.

Using acoustic rhinometry, both 50 and 100 mg PPA had a decongestive effect during the first 3 h after administration, but no significant difference was noted between the effects of the different concentrations of these drugs. Although, the decongestive effect of 100 mg PPA was significantly greater than placebo, no such difference was found between that of 50 mg PPA and placebo. A slight dose-response increase in the systolic and diastolic blood pressures occurred in the first 3 h after the administration of PPA with 100 mg PPA.

PPA 100 mg had the same decongestive effect as oxymetazoline nasal spray (0.1 ml in each nostril) without benzalkonium chloride (Nezeril®, Astra, Sweden 0.5 mg/ml www.astrazeneca.se).

The mean mucosal surface position (mm) as measured with rhinostereometry in 15 healthy subjects after administration of one dose of 50 mg, or 100 mg phenylpropanolamine or placebo. The zero level represents the reference position before starting the medication.
Paper II
Evaluation of a new method for assessing symptoms

Comparison of the symptoms estimated with the two methods, the visual analogue scale (VAS) and telephone scoring system showed strong correlations with nasal blockage, nasal secretions, sneezing, itching in the nose and total nasal discomfort. The symptom scores were also similar. The questionnaire about the two methods which the patients answered showed that the telephone method was easy or very easy to use. More than 80 percent of them preferred the telephone method, as a method for use in future studies.

The correlation between symptom scoring, for total nasal discomfort, by the telephone scoring method and the visual analogue scale (VAS) \( p < 0.0001, r = 0.9 \).
Paper III
Phenylpropanolamine’s decongestive effect on the nasal mucosa of pregnant women with nasal stuffiness

At the end of the study, the subjective symptoms of nasal stuffiness had improved significantly in the PPA group, but not in the placebo group. However, no significant difference was noted between the PPA and placebo groups at the end of the study.

Rhinostereometry showed no statistically significant difference between the decongestive effect of PPA and placebo.

During the study the systolic and diastolic blood pressures remained about the same and no adverse effects were reported.

every evening in 38 women with pregnancy rhinitis during treatment with either phenylpropanolamine (PPA) 50 mg twice daily or placebo. Date are presented as Mean ± SEM p < 0.001.
Paper IV
Evidence that nasal mucosal hyperreactivity in healthy women is induced by high levels of estrogen

With rhinostereometry we found no difference in the nasal mucosal swelling of the inferior turbinate between patients with low s-oestrogen (LE) and high s-oestrogen (HE) levels at baseline. Challenge with histamine (2 mg/ml) increased swelling in both groups. However, in the latter group, the swelling was significantly greater at 2 and 5 minutes. After 10 minutes, the mucosal swelling returned to baseline in both groups.

With laser-Doppler flowmetry the velocity of moving blood cells tended to be higher with high s-oestrogen levels at baseline. Challenge with histamine significantly increased the velocity in both groups at 2, 5 and 10 minutes. It was noteworthy, however, that the increase in velocity was greater at low s-oestrogen than at high s-oestrogen levels, evaluated as a systematic difference over 2,5 and 10 minutes.

At baseline, the perfusion tended to be higher with high s-oestrogen levels. Challenge with histamine significantly increased perfusion in both groups at 2, 5 and 10 minutes, but tended to be greater at low s-oestrogen than at high s-oestrogen levels.

Neither the estrogen levels nor histamine challenge affected the concentration of moving blood cells and we found no difference between low s-oestrogen and high s-oestrogen levels. No correlation was noted between individual estrogen levels and mucosal swelling or the microcirculation.

![Graph](image-url)

Changes in nasal mucosal swelling after histamine challenge in high s-oestrogen, HE (△) and low s-oestrogen, LE (●). Bars show 95% CI, the swelling was significantly greater for the HE treatment at 2 (p<0.001) and 5 (p=0.029) minutes. Estimated differences with 95% CI, at 2 and 5 minutes, 0.60 (0.34 - 0.86) and 0.25 (0.03 - 0.46).
Changes in velocity after histamine challenge in high s-estrogen, HE (▲) and low s-
estrogen, LE (●). Bars show 95% CI. LE was estimated to be systematically greater than 
HE measured over 2, 5 and 10 minutes (pH < 0.029). Estimated difference with 95% CI, 
62.1 (6.76 – 117.5).

Paper V
Oestrogen receptor ß in mast cells in the human nasal mucosa

Immunohistochemistry showed distinctly ER ß positive cells in all sections of the nasal mucosa 
from the patients. These cells, located subepithelially and close to the basal membrane, showed 
no relation to the vascular or glandular structures or to the epithelium. They were comparatively 
large with a centrally located nucleus. Immunoreactive signals for ER ß proteins were seen in 
their nuclei. Weak signals of ER ß immunostaining in the cytoplasm were also detected, which 
may indicate the presence of ER ß proteins in the cytoplasm. However, subglandular, endothelial 
and epithelial cells showed no immunoreactivity for ER ß in the nasal mucosa. The distribution 
of the cells was similar in men and women. With this immunohistochemical technique we could 
not detect any differences in the concentrations of ER ß in men and women or between 
individuals.

On the other hand, double immunostaining showed co-expression of ER ß with the specific mast 
cell marker, anti-human mast cell tryptase, in the vast majority of ER ß positive cells.
A. Double immunostaining micrographs showing co-expression of ER $\beta$ with the specific mast cell marker in the majority of the ER $\beta$ positive cells. The cells is located subepithelially and close to the basal membrane. Magnification x 25.

B. Magnification of double immunostaining micrographs showing co-expression of ER $\beta$ with the specific mast cell marker. Magnification x 100.

C. Magnification of immunostaining micrographs showing expression of ER $\beta$. Magnification x 100.

D. Magnification of immunostaining micrographs showing expression with the specific mast cell marker, anti-human mast cell tryptase. Magnification x 100.
Discussion

In Papers I and III, we found that the decongestive effect of phenylpropanolamine (PPA) 100 mg is excellent in healthy volunteers, as measured with rhinostereometry and acoustic rhinometry, and that of PPA 50 mg has a decongestive effect on nasal stuffiness in patients with pregnancy rhinitis, as measured by symptom scores. PPA is widely used as a nasal decongestant in patients with viral infections and sinusitis and many prefer it to topical decongestants. The patients usually obtain their prescriptions from general practitioners. Many ENT specialists do not believe that PPA is effective because of the lack of controlled clinical studies using objective measurements for assessment of the decongestive effects of the drug. Since no satisfactory therapy is available for pregnancy rhinitis, our results must be regarded as interesting and useful. No agreement exists, especially in the USA, as regards the possible deleterious effect of PPA on health. It was therefore PPA removed from the market, but then shown to have no significant clinical cardiovascular, euphoric or CNS reinforcing effects when given in recommended doses. No correlation was found between cough and cold mixtures containing PPA and haemorrhagic stroke. Some diet preparations contain PPA and a correlation between PPA and haemorrhagic stroke was reported in patients who were using those that suppress appetite. However, overweight is a known risk factor for haemorrhagic stroke. In our studies, we found no significant changes in blood pressure in women with pregnancy rhinitis who were given recommended doses. 100 mg PPA had an excellent decongestive effect in healthy subjects but they showed a slight increase in their blood pressure. Pregnancy is accompanied by an increase in blood volume and frequently by a decrease in blood pressure. It remains uncertain whether the decongestive effect of 100 mg PPA on the nasal mucosa occurs without influencing the blood pressure. Since no satisfactory treatment of pregnancy rhinitis is available, more studies are needed to elucidate the effects of this dose in women with pregnancy rhinitis.

Concern has been expressed that PPA may have a negative effect on foetal development because a few authors reported an association between the maternal use of PPA and gastroschisis and small-bowel atresia. Others found no significant association between the use of PPA and gastroschisis and small-bowel atresia, but in a later paper, they noted an interaction between vasoconstrictive drugs and maternal smoking, which they regarded as a risk for gastroschisis and small-bowel atresia. These were all retrospective case-control studies, and the possibility of recall bias exists.

In another report which included over 2000 women, PPA had no statistically significant effect on the total malformation rate of any specific malformation. This study was undertaken to assess delivery outcome -i.e., the presence of congenital malformations after maternal use of oral decongestants during pregnancy, and information on drug use that had been collected before the pregnancy outcome was known.

For purposes of research, it is essential to evaluate subjective complaints of nasal obstruction. This is usually done by using a symptom severity score rating or a visual analogue scale (VAS). In Paper II, we evaluated a new method for assessing symptoms with which the patients used their telephone to call a computer. Its great advantage is that the computer records the time when the estimate is made. In this study, we wanted the patient to score their symptoms in the morning and evening. Those who went to bed in the middle of the night and then woke up in the middle of the day could therefore be excluded afterwards. Today, most people have a cell phone and they use it frequently -e.g., for bank transactions, purchase of bus tickets, etc. In our opinion this is a good method for assessing symptoms in future studies. Since we found that telephone
Nasal congestion is difficult to quantify by clinical examination. Therefore objective assessment of the nasal airway is essential for rhinologic research. The fact that there are many methods available indicates that no method has proved good enough to replace all the others, but it also shows that several are suitable on different occasions.

Moreover, subjective complaints about nasal obstruction are not necessarily confirmed by objective measurements. For example, an increase in nasal stuffiness is not always accompanied by an objective increase in nasal airway resistance, a decrease in nasal peak flow or a reduction in the acoustic rhinometry value. Because sensations in humans are complex and not entirely linear, small changes in nasal shape, cause large shifts in the symptom severity score or VAS.

In Paper III, we showed that in a group of women with pregnancy rhinitis, PPA 50 mg twice daily had a decongestive effect on nasal stuffiness, as measured by the symptom score. However, the findings with rhinostereometry were inconclusive. It would have been useful to combine rhinostereometry with laser-Doppler flowmetry as we did in Paper IV, but the equipment was not available then.

In Paper I, we made measurements with rhinostereometry and acoustic rhinometry, both of which are sensitive methods for studying nasal mucosal swelling. This was done because rhinostereometry had hardly been used outside Sweden at that time. A acoustic rhinometry is well-known and has been evaluated by others. However, some differences between the findings were noted, which accords with those in another study that showed a poor but significant correlation between these methods. This is not surprising because rhinostereometry records swelling, in mm, from a defined point on the inferior concha, but acoustic rhinometry measures the minimal cross-sectional area in a defined plane, or volume, in a given region of the nose. Rhinostereometry has now been used in several studies and is well-established. Its great advantage is that even minor changes are easily seen and accurately measured. Moreover it can be combined with laser-Doppler flowmetry (LDF), which is the only non-invasive method for studying the microcirculation. This combination permits the use of two non-invasive methods for direct and simultaneous measurements of congestion and the microcirculation because rhinostereometry allows the laser probe to be in place in the nose during the measurements of congestion (Paper IV). A disadvantage is that the investigator must be experienced in its use. In Paper III, PPA 50 mg twice daily and placebo had a decongestive effect on the nasal mucosa, as measured by rhinostereometry. This may seem surprising. However, the method is very sensitive and the measurements require an acclimatization period of about 30 minutes before the nasal mucosa reaches a baseline position, which is not affected by physical stress, temperature, etc. In this study, the acclimatization periods may have been less than 30 minutes because these pregnant women were in a hurry. It seems possible that higher concentrations of adrenalin and noradrenalin have a decongestive effect on the nasal mucosa.

The exact cause and pathophysiology of pregnancy rhinitis remain to be elucidated. Hormonal levels, especially those of oestrogen may induce nasal congestion. Some data support the view that the rhinitis is due to higher levels of oestrogen, e.g., wheal-flare reactions in the skin test increase at the mid-menstrual cycle when oestrogen levels peak, and women on contraceptives that contain high levels of oestrogen frequently have nasal complaints. However, pregnancy rhinitis, has few distinct rhinoscopic signs and the possibility exists that the nose is not congested but hyperreactive.
In Paper IV, we showed that the increase in oestrogen levels during in vitro fertilization (IVF) in healthy women causes nasal mucosa hyperreactivity after histamine challenge. However, no increase in nasal mucosal swelling was noted with low and high oestrogen levels before histamine challenge although perfusion and velocity tended to increase at high oestrogen levels. We also found that the LDF measurements of increases in perfusion and velocity after histamine challenge were lower at high than low oestrogen levels. Similar results were seen in patients with allergy as compared to those without. The increases in perfusion and velocity were lower in the former group.

To study the role of oestrogen in pregnant women is difficult because of the effects and variations in the concentrations of other hormones. We therefore recruited healthy women who were undergoing in vitro fertilization (IVF). In our model, we increased their levels of oestrogen markedly for only 12-14 days and noted no measurable nasal swelling at baseline. In contrast, patients with pregnancy rhinitis develop nasal congestion, but they have high oestrogen levels over a long period with resultant hyperreactivity, which may also cause this symptom. Although several mechanisms can explain pregnancy rhinitis, it seems likely that oestrogen is involved.

In Paper V, we found that immunoreactivity for monoclonal antibodies to ER ß is present in the subepithelium of the nasal mucosa. Moreover, it may be located in mast cells, but not in the glands or vascular structures.

Mast cells are best known for their potent effector functions in allergic disorders. In recent years, however, mast cells have been shown to be involved in a surprisingly complex range of immune functions that extend far beyond allergies and include the development of autoimmune disorders and the initiation and maintenance of adaptive and innate host responses. In normal pregnancy, the histamine levels in blood may be highest during the first trimester, but are usually lower during the second and third trimesters than in healthy subjects who are not pregnant. Many of the complications of pregnancy are associated with increases in the levels of histamine in blood. More data are needed on the possible roles of histamine in normal and complicated pregnancies, but at present it seems likely that treatment to correct high histamine levels may be of value in pregnancy. The role of oestrogen in mast cells and the release of histamine are not clear.

Antihistamines are effective in allergy, a disease mediated by the release of histamine from mast cells. Very few studies have been done on the treatment of pregnancy rhinitis with antihistamines. The second generation antihistamines have not been associated with an increase in congenital malformations in humans; however, most of them lack large safety studies in pregnancy.

Intranasal sodium cromoglycate is a mast cell stabiliser used in allergic rhinitis, but no data are available on the treatment of pregnancy rhinitis with this drug either. In view of the above, the relations between oestrogen, mast cells, histamine and pregnancy rhinitis need to be clarified.

In conclusion, PPA in double the recommended dose has an excellent decongestive effect on the nasal mucosa of healthy subjects, and it has the same decongestive effect as oxymetazoline nasal spray. PPA given in the recommended dose relieves nasal stuffiness in women with pregnancy rhinitis and has no effect on blood pressure. These data indicate that PPA may be useful in the treatment of pregnancy rhinitis. In the human nasal mucosa of healthy subjects, we found oestrogen receptor(ER) ß positive cells. The increase in oestrogen levels in blood during in vitro fertilization (IVF) in a group of healthy women causes hyperreactivity of the nasal mucosa after histamine challenge. These findings indicate that oestrogen may play a role in the development of pregnancy rhinitis.
SUMMARY

Phenylpropanolamine (PPA) in double the recommended dose - i.e., 100 mg has an excellent decongestive effect on the nasal mucosa of healthy subjects, but the systolic and diastolic blood pressures also increase temporarily.

The effect of PPA on the nasal mucosa of healthy subjects is about the same as that of oxymetazoline nasal spray (0.5 mg/ml; 0.1 ml in each nostril) without benzalkonium chloride.

When PPA is given in the recommended dose - i.e., 50 mg twice daily - it relieves nasal stuffiness in pregnancy rhinitis and has no effect on blood pressure.

The telephone scoring of symptoms is a reliable and adequate method for recording symptoms scoring as hard data.

Rhinostereometry shows that swelling of the nasal mucosa increases after histamine challenge in women with high levels of oestrogen in their blood during in vitro fertilization.

Laser-Doppler flowmetry shows that the microcirculatory parameters - i.e., velocity of moving blood cells and perfusion increase less when the oestrogen levels in blood are high.

With immunocytotoxicity, we present evidence for the presence of estrogen receptor β in the nasal mucosa of healthy men and women. In addition, using double staining we show that the immunoreactivity is mainly located in subepithelial mast cells.
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Pregnancy rhinitis: pathophysiologcal effects of oestrogen and treatment with oral decongestants

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