Changes in progesterone receptor mRNA content in the rabbit lung during early pregnancy and after sex steroid hormone treatment

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Abstract

In this work we determined progesterone receptor (PR) mRNA content in female rabbit lung during the first 5 days of pregnancy and in ovariectomized animals after subcutaneous injection of oestradiol benzoate (25 µg/kg) for 2 days or oestradiol benzoate (25 µg/kg) for 2 days plus a single dose of progesterone (5 mg/kg) on day three. On each day (0–5) of pregnancy and 24 h after the last dose in the case of the treated animals, animals were killed and lung was excised; total RNA was extracted and processed for Northern blot analysis. The results showed three main PR mRNA transcripts (6.1, 4.4 and 1.8 kb) in rabbit lung.

The 4.4 kb species was the most abundant. PR mRNA content was markedly increased by oestradiol benzoate and downregulated by progesterone. It significantly increased on the first day of pregnancy and then diminished progressively, reaching its lowest value on day 5. These findings suggest that PR mRNA content in the rabbit lung is regulated by sex steroid hormones and changes according to the physiological concentrations of oestradiol and progesterone.

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Introduction

Progesterone participates in the regulation of respiratory function in mammals. This sex steroid hormone mediates the increase in ventilation and the reduction in alveolar carbon dioxide tension observed in the luteal phase of the menstrual cycle and during pregnancy in humans (Goodland & Pommerenke 1952, Goodland *et al.* 1954). Administration of progesterone to several species including humans induces hyperventilation (Hosenpud *et al.* 1983, Regensteiner *et al.* 1989, Tatsumi *et al.* 1991).

Most actions of progesterone are mediated by specific intracellular progesterone receptors (PRs), which in various tissues are upregulated by oestradiol and downregulated by progesterone (Kraus & Katzenellenbogen 1993, Camacho-Arroyo *et al.* 1996). It has been shown that progestins can stimulate ventilation in the female rat only when uterine PR concentrations are increased by previous administration of oestradiol (Brodeur *et al.* 1986).

PRs have been located by immunohistochemistry in several lung cell types such as alveolar epithelial cells, interstitial, endothelial and muscle cells (Camacho-Arroyo *et al.* 1994). It was found that oestradiol treatment of ovariectomized rabbits increased the number of PR-immunoreactive cells in the lung compared with that in ovariectomized animals treated with vehicle (Camacho-Arroyo *et al.* 1994). In the present study, we have

evaluated the role of oestradiol and progesterone in the regulation of PR mRNA content in rabbit lung and its variations during early pregnancy.

Materials and Methods

Animals and treatments

Adult female New Zealand White rabbits (3–4 kg) maintained under a 12:12 h light/darkness cycle with food and water available *ad libitum* were used in this study. Twelve animals were ovariectomized under ketamine (Rhône Mérieux, Querétaro, México) anaesthesia (80 mg/kg). Two weeks later they were randomly assigned to the following s.c. treatments (four animals/treatment): (a) daily administration of oestradiol benzoate (OB) (25 µg/kg) for 2 consecutive days, (b) the above described administration of OB followed by progesterone (5 mg/kg) on day 3, and (c) vehicle alone (corn oil). At 24 h after the last dose, the animals were deeply anaesthetized with ketamine, and the lung and uterus were dissected out onto ice and immediately processed for total RNA extraction.

In another experiment intact female rabbits were mated twice on the same day with an experienced buck. The day of mating was designated day 0. Animals (four/group) were deeply anaesthetized with ketamine and killed 4, 24,

48, 72, 96 and 120 h (days 0–5) after the second coitus, the lung was removed onto ice and processed for RNA extraction.

Northern blot analysis

Total RNA was obtained from each tissue by the single-step method based on guanidinium isothiocyanate–phenol–chloroform extraction (Chomczynski & Sacchi 1987) using Trizol reagent (Gibco-BRL, Inc, Gaithersburg, MD, USA). In all cases RNA extracted from each animal was individually analysed. RNA concentration was determined by measurement of absorbance at 260 nm. Total RNA samples (25 µg) were size-fractionated by electrophoresis on 1.1% agarose gels in the presence of 2.2 M formaldehyde and then transferred to GeneScreen membranes (New England Nuclear, Boston, MA, USA). Blotted RNAs were hybridized with [α - 32 P]dCTP PR cDNA probe labelled by the random primer method (Feinberg & Vogelstein 1983). After hybridization, membranes were washed twice with $2 \times$ SSC at room temperature for 15 min, followed by $0.1 \times$ SSC/ 0.1% SDS at 56°C for 45 min. The membranes were then exposed to Hyperfilm β max films (Amersham Co, Amersham, Bucks, UK) for 72 h at -70°C . After exposure, membranes were stripped and rehybridized with [α - 32 P]dCTP-labelled cyclophilin 18 PCR product probe obtained in our laboratory (Díaz-Sánchez *et al.* 1995). Cyclophilin 18 (Harding *et al.* 1986) was used as a constitutive gene expression control (Haendler *et al.* 1987). The intensity of the bands in the autoradiographs was quantified by densitometry using a Scan jet 3C apparatus (Hewlett-Packard, CA, USA). Data for PR mRNA content during early pregnancy were corrected using data for cyclophilin and analysed using one-way ANOVA followed by Student's *t*-test. The Epistat statistic program (Epistat Services, TX, USA) was used to calculate probability values.

Results

Northern blot analysis showed the presence of three main PR mRNA transcripts of 6.1, 4.4 and 1.8 kb in rabbit lung. The 4.4 kb species was the most abundant transcript (Figs 1 and 2). The content of each PR mRNA transcript changed according to the steroid hormonal environment of the animals. Thus we observed that the treatment of ovariectomized rabbits with OB induced a marked increase in PR mRNA content in the lung whereas the administration of progesterone after OB priming reduced it (Fig. 1). These effects were more evident in the 4.4 kb PR mRNA transcript than in the others. Interestingly, the largest PR mRNA transcript (6.1 kb) was detected in the uterus but not in the lung of ovariectomized animals (Fig. 1). This PR mRNA transcript was clearly observed in the lung on day 1 of pregnancy (Fig. 2).

Figure 1 A representative Northern blot of PR mRNA from rabbit lung. Ovariectomized rabbits were treated with vehicle (V), OB (E) or OB+progesterone (P) as described in Materials and Methods. Total RNA (25 µg) was fractionated on 1.1% agarose gels, and transferred to GeneScreen membranes which were then hybridized with both [α - 32 P]PR cDNA and cyclophilin (CP) probes and exposed for 72 h using Hyperfilm β max films. CP was used as a constitutive gene expression control. Lane U shows PR mRNA from the uterus of ovariectomized rabbits treated with OB. The main PR mRNA transcripts (6.1, 4.4 and 1.8 kb) are indicated.

During early pregnancy, PR mRNA content markedly changed in rabbit lung (Fig. 2). A significant increase in PR mRNA content was found on the first day of pregnancy; it then diminished progressively until it reached its lowest value on day 5, as shown by the PR:cyclophilin ratio determined by densitometric analysis (Fig. 3).

Discussion

In this study we determined the presence of PR mRNA in rabbit lung as well as changes in its content after oestradiol and progesterone treatment and during early pregnancy. Several PR gene transcripts were found in rabbit lung, as has also been observed in the uterus of rabbit and rat (Gutiérrez-Sagal *et al.* 1993, Kraus & Katzenellenbogen 1993). We observed three main PR transcripts (6.1, 4.4 and 1.8 kb) in the lung. The 4.4 kb PR mRNA species was the most abundant. The size of this transcript is similar to the main PR mRNA species reported in the chicken (Jeltsch *et al.* 1990), while the largest one (6.1 kb) corresponds to the transcript previously described in rabbit uterus (Loosfelt *et al.* 1986, Misrahi *et al.* 1988).

Figure 2 A representative Northern blot of PR mRNA from the lung of rabbits during early pregnancy. Total RNA (25 µg) from the lung of early pregnant rabbits (days 0–5) was used for Northern blot analysis which was performed as indicated in Fig. 1. Cyclophilin (CP) was used as a constitutive gene expression control. The three main PR mRNA transcripts (6·1, 4·4 and 1·8 kb) found in the lung are indicated.

Figure 3 Densitometric analysis of PR mRNA content in the lung of early pregnant rabbits. PR mRNA content in rabbit lung during early pregnancy was corrected by using data for the constitutively expressed cyclophilin (CP) gene on days 0–5 of pregnancy. Results are expressed as mean $\pm$ S.E.M. from four animals. *** $P < 0.05$ compared with the other days of pregnancy.

In ovariectomized rabbits, the 6·1 kb PR transcript was only observed in the uterus and not in the lung (Fig. 1). However, this transcript was clearly detected in the lung of pregnant rabbits on day 1 (Fig. 2). The functional relevance of the presence of several PR transcripts in the lung is not known since, according to Loosfelt *et al.* (1984), only the PR B isoform is expressed in the rabbit, although the possibility of the presence of two PR isoforms in rabbit lung cannot be discounted.

We found that the relative abundance of each PR mRNA transcript in the lung changed according to the hormonal status of the animals. PR gene expression in the lung was upregulated by oestradiol and downregulated by progesterone in a manner similar to that observed in the uterus and the hypothalamus of the rabbit (Camacho-Arroyo *et al.* 1996). The effects of oestradiol and progesterone on PR gene expression may be due to changes in the rate of PR transcription or mRNA stability (Turcotte *et al.* 1991). PR gene expression in the lung could be up- and down-regulated by oestradiol and progesterone respectively via the oestrogen responsive element which overlaps the initiation site of transcription of the PR rabbit gene (Savouret *et al.* 1991).

Our results on the regulation of PR gene expression by sex steroid hormones assessed by PR mRNA content in the lung are in line with data obtained at the protein level by immunohistochemical examination (Camacho-Arroyo *et al.* 1994). It has been reported that oestradiol treatment of ovariectomized rabbits increased the number of PR-immunoreactive cells in the lung as compared with that of ovariectomized animals treated with vehicle alone (Camacho-Arroyo *et al.* 1994). There is a correlation between the effects of oestradiol on PR gene expression at both the mRNA and protein levels, suggesting that the changes in PR protein content in rabbit lung after sex steroid hormone treatment are the result of modifications at the transcriptional level.

During early pregnancy, PR mRNA was detected in rabbit lung on day 0, with a marked increase on day 1 followed by a clear diminution on days 2–5 (Figs 2 and 3). This PR gene expression pattern was similar to that found in rabbit endometrium, although in the latter the highest PR gene expression was found on day 2; on day 3 PR mRNA content was still high and diminished until days 4 and 5 (Gutierrez-Sagal *et al.* 1993). These differences are probably due to the presence of tissue-specific factors that regulate PR gene expression differently in the uterus and lung. Interestingly, in the case of the uterus, no correlation was found between PR mRNA and PR protein content: on day 3 of pregnancy endometrial PR protein was not detectable in spite of the high PR mRNA content (Gutierrez-Sagal *et al.* 1993). Although we observed a correlation between the effects of oestradiol on PR mRNA content and PR protein levels (Camacho-Arroyo *et al.* 1994) in the lung, it is necessary to determine whether this correlation is maintained during early pregnancy in order to understand other aspects of PR gene expression regulation and the physiological meaning of PR changes in the lung during early pregnancy.

Our results suggest that, under physiological conditions, PR mRNA content in the lung depends on the concentration of sex steroid hormones. Maximal PR mRNA content was observed on day 1 of pregnancy when plasma progesterone concentration is low, whereas the lowest PR

mRNA content occurred on day 5 after a marked increase in plasma progesterone concentration (Challis *et al.* 1973, Hilliard & Eaton 1971).

Although it has been shown that progesterone can increase the content of flavin-containing mono-oxygenase isoform B in rabbit lung (Lee *et al.* 1993) and decrease both β -adrenergic receptor activity and cAMP levels (Moawad *et al.* 1982, Nabishah *et al.* 1992), hitherto the molecules regulated by progesterone via PR activation, which are involved in the increase in ventilation and the reduction in alveolar carbon dioxide tension observed during pregnancy, are not known and deserve further investigation.

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