Molecular Human Reproduction Vol.7, No.9 pp. 875–879, 2001

Interactions between progesterone receptor isoforms in myometrial cells in human labour

Doris Pieber[1], Victoria C.Allport[2], Frank Hills[2], Mark Johnson[3] and Phillip R.Bennett[2,4]

[1]Department of Obstetrics and Gynaecology, Karl-Franzens University Graz, Austria, [2]Imperial College School of Medicine Parturition Research Group, Institute of Reproductive and Developmental Biology, Hammersmith Hospital, Du Cane Road, London W12 and [3]Department of Maternal & Fetal Medicine, Imperial College School of Medicine, Chelsea and Westminster Hospital, London, UK

[4]To whom correspondence should be addressed at: Division of Paediatrics, Obstetrics and Gynaecology, Institute of Obstetrics and Gynaecology, Imperial College School of Medicine, Queen Charlotte's and Chelsea Hospital, Ducane Road London W12 0HS, UK. E-mail: pbennett@ic.ac.uk

Progesterone acts to maintain uterine quiescence during pregnancy. In contrast to many other species, no decrease in maternal serum levels of progesterone can be observed in humans before the onset of labour. Therefore, a 'functional' progesterone withdrawal in association with labour has been proposed. In humans the progesterone receptor (PR) exists in two isoforms, PR-A and PR-B. While PR-B generally mediates the effects of progesterone upon gene transcription, the role of PR-A during pregnancy, and in parturition, is unknown. In this study, term myometrium cells cultured before the onset of labour were transiently transfected with expression vectors for either PR-A or PR-B. Only those cells expressing PR-B significantly increased expression of a progesterone-sensitive reporter when stimulated with progesterone. Co-transfection of both isoforms of PR demonstrated that PR-A is a dominant repressor of transactivation in these cells. Western blot analysis showed that PR-A is present in human myometrium samples taken only after, but not before, the onset of labour. These data suggest that increased expression of PR-A in human myometrium may contribute to 'functional' progesterone withdrawal and the initiation of human labour.

Key words: labour/myometrium/parturition/progesterone receptor

Introduction

For more than 60 years, the role of the 'pro-pregnancy' hormone, progesterone, as an inhibitor of uterine contraction has been recognized. In 1965 Csapo referred to the 'progesterone block' which opposes the excitatory effect of oestrogen, prevents myometrial responsiveness to uterotonic agents and allows pregnancy maintenance (Csapo and Pinto-Dantas, 1965). For labour to occur, however, this 'progesterone block' must be overcome. In most species, there is a detectable decrease in the maternal progesterone serum concentration shortly before the onset of labour. In a few species, including humans, this is not the case. In different species, one of three distinct mechanisms of progesterone withdrawal occurs. Firstly, in many species including rats, mice, rabbits, goats and pigs, the corpus luteum is the main source of progesterone until term, when its regression, luteolysis, directly causes progesterone withdrawal and labour onset follows. Secondly, in sheep, an increase in fetal cortisol induces the activity of the enzyme 17α-hydroxylase which converts progesterone into oestrogen (Challis *et al*., 1995). There is therefore an increase in oestrogen and decrease in progesterone. The third mechanism is seen in guinea-pigs, non-human primates and humans. Luteolysis occurs early in gestation and progesterone production is then from the placenta. There is no placental expression of 17α-hydroxylase, so shifts in steroidogenesis, as seen in sheep, do not occur. Progesterone is, however, apparently involved in pregnancy maintenance as administration of anti-progestins, such as RU-486 and epostane, cause a considerable increase in uterine contractility, abortion in humans in the first and second trimester and premature labour in macaques (Haluska *et al*., 1994). There is also an associated increase in the expression of a range of 'pro-labour' genes which are normally repressed by the presence of progesterone (Lye, 1994). It therefore follows that in these species a third, 'functional' progesterone withdrawal must occur.

Several explanations for 'functional' progesterone withdrawal have been proposed, such as loss of progesterone receptors (PR) or a change in receptor isoform expression, binding of progesterone to a high affinity protein and therefore a reduction in free active steroid (Westphal *et al*., 1977;

McGarrigle and Lachelin, 1984), production of a local anti-progestin, sequestration of progesterone into lipoproteins or localization of the progesterone withdrawal to the avascular fetal membranes so that a decrease is not detected in the circulation (Mitchell *et al*., 1993). An increase in progesterone metabolism has also been suggested as has the competitive binding of both progesterone and cortisol for the same receptor, where increasing cortisol concentrations simply out-compete progesterone (Karalis *et al*., 1996). This theory requires there to be no expression of the PR, and for progesterone to be acting instead through binding to the glucocorticoid receptor, which seems unlikely given that there is PR expression in the uterus (Rezapour *et al*., 1997). None of these hypotheses has been established, and so the question remains, how does 'functional withdrawal' of progesterone prior to human labour occur?

PR is a member of the nuclear receptor superfamily. In the absence of ligand, the receptor is transcriptionally inactive. However, with the binding of ligand the monomeric receptor undergoes a conformational change and is activated. PR dimers can then act as a transcription factor regulating the transcription of genes containing a DNA binding site for PR, i.e. progesterone response element (PRE).

PR exhibits the common structure of all nuclear hormones, containing a DNA-binding domain (DBD), a hormone-binding domain (HBD) and a variable N-terminal domain. PR is unique within its family group as it exists as two isoforms, PR-B (116 kDa) and PR-A (94 kDa). PR-A is a truncated form of PR-B lacking the first 164 N-terminal amino acids. Two distinct promoters within the single copy gene for PR have been shown to independently regulate the expression of the two isoforms of PR (Kastner *et al*., 1990). Antibodies have been generated to the unique portion of PR-B and are therefore specific for that isoform. Since there is no part of PR-A which is unique, no specific PR-A antibody is available (Clarke *et al*., 1987).

Both PR isoforms contain activation function elements (AF) through which they interact with and regulate the basic transcriptional machinery required for gene expression. AF1 is located 90 amino acids upstream of the DBD and AF2 is found within the HBD and both are common to both isoforms. However, AF3 is found in the B upstream sequence (BUS) located in the N-terminal region that is present only in PR-B. AF3 is therefore missing from PR-A (Sartorius *et al*., 1994).

The expression of pure homo-dimers of PR-B has shown that this receptor functions as an activator of transcription. Pure PR-A act as a repressor of transcription (Vegeto *et al*., 1993; Mohamed *et al*., 1994). An inhibitory function region (IF) has also been characterized as lying in a 292 amino acid segment upstream of AF1. IF inhibits the activation of transcription directed by both AF1 and AF2 but does not affect AF3 (Hovland *et al*., 1998). This may explain the functional difference between these two receptor isoforms. Heterodimers of PR-A/B also function as transcriptional repressors, therefore PR-A is a dominant negative inhibitor of the transcriptional regulation due to PR-B (Sartorius *et al*., 1994). PR-A has also been shown to inhibit the transcriptional activity of other steroid receptor family members (Vegeto *et al*., 1993). It is likely that it is the relative expression of the two isoforms of PR in target cells, rather than simply their presence or absence, which is responsible for the biological response of that tissue to progesterone.

Studies of the expression of PR isoforms in uterine tissues have produced conflicting results. Various authors have demonstrated PR expression in the decidua and myometrium (Khan-Dawood and Dawood, 1984; Padayachi *et al*., 1990; How *et al*., 1995; Rezapour *et al*., 1997). However, this expression has, in some cases, been found to decrease towards term (How *et al*., 1995). Others have found no change in receptor expression (Rezapour *et al*., 1997). One group was unable to find expression of PR in trophoblast at term (Karalis *et al*., 1996), but PR expression has been demonstrated in the human fetoplacental vascular tree (Cudeville *et al*., 2000). Although definitive data concerning the expression of PR isoforms in uterine tissue remains to be elucidated, it is clear that the phase of pregnancy maintenance and myometrial quiescence is, biochemically, a time of active repression of genes by progesterone that would otherwise drive the uterus into labour.

Here we have used transfection studies to examine transactivation due to the individual and combined expression of both isoforms of PR in human myometrial cells and we have examined PR isoform expression in myometrial tissue taken before and after the onset of human labour at term.

Materials and methods

Progesterone was purchased from Sigma (Poole, UK), and dissolved in ethanol. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma), supplemented with 10% fetal bovine serum (FBS; Sigma), 100 IU/ml penicillin (Life Technologies, Paisley, UK), 100 μg/ml streptomycin (Life Technologies) and 2 mmol/l L-glutamine (Life Technologies). Protein extraction was performed using T-wash solution (50 mmol/l Tris–buffer, 10 mmol/l EDTA, 1% Triton-100) containing proteinase-inhibitors (Sigma).

Myometrial cell culture

Myometrial tissue was taken from the lower uterine segment at the time of elective Caesarean section after informed consent as approved by our local ethics committee. The tissue was cut into slices (2×10×10 mm) and incubated in Dispase grade II (Life Technologies) at a concentration of 10 g/l in Hanks' balanced salt solution (HBSS; Life Technologies) for 90 min at 37°C with gentle shaking in a water-bath. The tissue was then incubated in fresh Dispase II solution for a further 90 min at 37°C. The tissue pieces were then washed three times in HBSS without calcium, magnesium or Phenol red before being placed in 50 ml of HBSS containing 240 IU/ml collagenase type II (Worthington Biochemical Corporation, Lakewood, NJ, USA), 2 IU/ml elastase (Sigma), 30 IU/ml deoxyribonuclease type I (Sigma) and 8 mg/ml bovine serum albumin and incubated at 37°C in a water-bath with gentle shaking for 90 min. This digestion process was repeated in fresh pre-warmed collagenase/elastase/DNase solution for 30 min at 37°C and all medium was retained for subsequent cell retrieval. The tissue pieces were gently massaged using a large Pasteur pipette after 15 min. Then all medium was filtered through a 250 μm sterile gauze and centrifuged at 1500 *g* for 6 min. The cells were washed in DMEM supplemented with 10% FBS, 100 IU/ml penicillin, 100 μg/ml streptomycin and 2 mmol/l L-glutamine for 2 min before repeated centrifugation and resuspension. Cell culture was performed in supplemented DMEM as above, in culture flasks. Cells were plated

into 24-well tissue culture plates at ~3×10^5 cells/well and allowed to grow to 80–90% confluence prior to experimentation.

Transient transfection

Tfx-50 (Promega, Southampton, UK) was prepared according to manufacturer's instructions and then left at –20°C for at least 16 h before use. The transfection procedure was performed according to instructions using a charge ratio of 3:1, 1 μg DNA/well and 2 h incubation time. To investigate the effect of PR-A and PR-B over-expression on progesterone-responsive genes, myometrial cells were transiently transfected with 0.5 μg of PR-A, PR-B or an empty expression vector and 0.5 μg of the MMTV-luciferase reporter construct. This construct contains the promoter of MMTV, which is highly progesterone sensitive, linked to the luciferase reporter. It acts to report progesterone-stimulated transactivational activity. To assess the repressive effect of PR-A on PR-B-mediated reporter expression, 0.5 μg PR-B and increasing amounts of PR-A (0.00, 0.02, 0.05, 0.1 or 0.2 μg/well) were co-transfected with the reporter vector. An empty vector was used to maintain a constant concentration of transfected DNA. Cells were cultured in serum containing media for 24 h, then washed and cultured for a further 24 h in serum and Phenol red-free media. Treatments were then performed in triplicate for the following 24 h. After treatment, the medium was removed and the cells were incubated with 200 μl of reporter lysis buffer (Promega) at room temperature on a horizontal shaker for 15 min. Lysates were then transferred to microcentrifuge tubes and stored at –80°C until analysis.

Luciferase assay

Luciferase assay buffer (Promega, Southampton, UK) was prepared according to the manufacturer's instructions. All samples and solutions were allowed to reach room temperature before analysis. Samples were centrifuged at 13 000 *g* for 1 min. 20 μl of the supernatant and 40 μl of the luciferase assay reagent (Promega) were mixed and luciferase activity was measured in a luminometer (Turner Design TD 20/20; Promega).

Western blots

Western analysis for PR-A and PR-B was performed using myometrial tissue which had been obtained from (i) elective Caesarean section before labour, (ii) emergency section because of fetal distress or (iii) emergency section due to failure to progress. T47D cell protein was used as the positive control for Western analysis. Tissue samples were placed into ice-cold T-wash containing proteinase inhibitors, homogenized for 30 s, incubated on ice for 30 min and then centrifuged at 1000 *g* for 10 min. The supernatant was transferred to a fresh tube and total protein measured (DC Protein assay; Bio Rad, Herts, UK). 10 μg of protein was made up to a total volume of 10 μl with T-wash and an equal volume of SDS-loading buffer (Sigma) added to each sample. Samples were then loaded on a 10% acrylamide gel and run at 140 V for 120 min. After separation, proteins were transferred by electrophoresis to a nitrocellulose membrane (Hybond ECL; Amersham Life Science) at 100 V for 1 h. Membranes were blocked in a 5% milk protein solution at room temperature for 1 h then washed and hybridized with the mouse anti-human PR monoclonal antibody (AB-52; Santa Cruz, Santa Barbara, USA), which recognizes both PR-A and PR-B, at 4°C overnight. Membranes were washed and incubated with the secondary antibody, a goat anti-mouse horseradish peroxidase-labelled polyclonal antibody (Santa Cruz) at room temperature for 1 h then washed again prior to detection. The ECL reagent (ICN Pharmaceuticals, Basingstoke, Hants, UK) was applied and the blot exposed to a radiographic film.

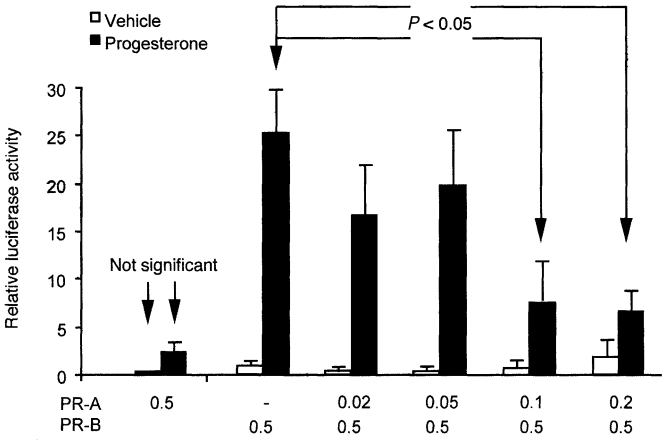

Figure 1. Transcriptional activity of progesterone receptor A (PR-A) and PR-A-mediated repression of PR-B transcriptional activity in human term myometrial cells. Primary cultures of non-laboured myometrial cells were transiently transfected with 0.5 μg DNA/well of an expression vector for PR-A or with increasing concentrations (0.02, 0.05, 0.1 or 0.2 μg DNA/well) of the expression vector for PR-A with simultaneous transfection of an expression vector for PR-B (0.5 μg DNA/well). Cells transfected with an empty expression vector served as controls. Transcription of the MMTV-luciferase reporter (0.5 μg DNA/well) was activated with progesterone (10 mmol/l), while its vehicle was used to determine basal reporter activity. Progesterone induced a minor, ~7-fold, increase in reporter activity in PR-A-transfected cells. In contrast, PR-B-expressing cells responded very effectively, with a 25-fold increase in reporter activity, to PR-B, and this effect was dose-dependently inhibited by co-transfection of cells with PR-A. Data are shown as means ± SEM, $n = 4$.

Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Fisher's least significance difference test. Probability values of $P < 0.05$ were considered to be statistically significant.

Results

Myocytes co-transfected with expression vectors for PR-A or PR-B and the progesterone-responsive MMTV-luciferase reporter vector (MMTVLuc) were stimulated with either vehicle or 10 mmol/l progesterone (Figure 1). Progesterone treatment did not have a significant effect on reporter expression in control cells co-transfected with an empty expression vector (data not shown). Progesterone treatment of cells co-transfected with PR-A and MMTVLuc caused a consistent, small increase in reporter expression but this did not achieve statistical significance compared to the control. However, progesterone treatment of cells co-transfected with the PR-B isoform significantly increased reporter expression (~25-fold) compared to control cells and those treated with vehicle (Figure 1).

To study the effect of PR-A expression on PR-B-mediated reporter expression, myocytes were co-transfected with 0.5 μg/well of the PR-B expression vector and increasing amounts of the PR-A expression vector (0.02, 0.05, 0.1 or 0.2 μg/well). The MMTVLuc construct (0.5 μg/well) was again used as the reporter. Co-transfection of increasing amounts of the PR-A expression vector resulted in a dose-dependent repression of reporter expression in the presence

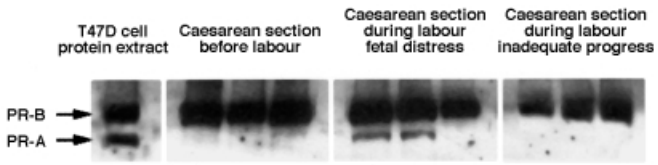

Figure 2. Western blot of term myometrium (10 µg total protein/lane) with a single monoclonal antibody directed against both PR-A (94 kDa) and PR-B (116 kDa). Sample 1 is protein from the T47D cell line which expresses both PR-A and PR-B. Samples 2–4 are from three different non-laboured myometrial samples (elective Caesarean section). In these samples only one band, PR-B, is present. Samples 5–7 represent three different samples of myometrium collected at emergency section for fetal distress with ongoing labour. PR-A expression is seen in two of the three samples. Samples 8–10 represent three different samples of myometrium collected at emergency section for failure for labour to progress. No expression of PR-A is seen in these samples.

of progesterone (Figure 1). Maximal repression of reporter expression was seen when PR-A was co-transfected at a concentration of 0.2 µg/well. Reporter expression in these cells in the presence of progesterone was reduced to 25% of that in cells without PR-A (Figure 1).

The expression of both the PR-A and PR-B isoforms was investigated using non-laboured myometrium samples from woman undergoing elective Caesarean section at term (n = 6), or from emergency Caesarean section for failure to progress (n = 3), and in myometrium from woman in whom labour had begun but required an emergency section due to fetal distress (n = 3) (Figure 2). PR-B (116 kDa) was apparently equally expressed in both non-laboured and laboured myometrium. However, PR-A (94 kDa) was only expressed in two of the three myometrial samples taken at Caesarean section during effective labour where there was fetal distress. PR-A expression was not found in myometrium from prelabour Caesarean section or from those women who underwent a Caesarean section for ineffective labour.

Discussion

These data demonstrate that the two isoforms of the PR do not function in the same manner in response to progesterone treatment in myometrial cells. Reporter expression was significantly induced by the presence of progesterone when the PR-B isoform was over-expressed but not when there was excess PR-A. PR-A may function as a weak activator of transcription as there was a slight rise in reporter expression in these cells. These findings are consistent with those of others. Vegeto *et al*. (1993) reported that PR-B is transcriptionally active in several cell types, whereas PR-A can act as either an activator or repressor of transcription depending on the cell in which it is expressed (Vegeto *et al*., 1993).

Co-transfection studies of both isoforms of PR in myometrial cells demonstrated that simultaneous expression causes a reduction in transcription from the progesterone-sensitive promoter in myometrial cells compared to that seen with PR-B alone. It is therefore assumed that PR-A causes repression of PR-B-mediated reporter expression. This repression was not simply due to an excess of PR-A as significant repression of transcription was achieved when a 5:1 ratio of PR-B to PR-A was used for transfection. PR-A is therefore a dominant repressor of PR-B transactivation in myometrial cells.

Although the molecular basis for the repression of PR-B by PR-A remains unknown, it may have an important functional significance. In the case of parturition, a slight increase in the expression of PR-A at term would cause repression of the transcriptional effects of PR-B and thus may be a cause of 'functional' progesterone withdrawal and labour onset. We have shown that in myometrium prior to the onset of labour there is no expression of PR-A. In two out of three myometrial samples collected at emergency section for fetal distress where labour was effective, PR-A was expressed, suggesting that the expression of PR-A is required for effective labour. Densitometric analysis of the Western blot showed that the ratio of PR-B:PR-A expression in laboured myometrial tissue was similar to that required for maximal inhibition of PR-B activity in the transfection studies (2:1 protein expression and 2.5:1 in co-transfection studies). These data are from small numbers of patients and a much larger study will be required to determine the degree to which increased PR-A expression correlates with labour.

In summary, we have demonstrated a difference in the function of the two isoforms of the PR in myometrial cells. We have shown that expression of the PR-A isoform has a dominant repressive effect on transcription of progesterone-sensitive genes within myometrial cells and that PR-A is expressed at this level in myometrium in patients who are in a state of effective labour at term. We therefore suggest that the PR-B isoform is expressed throughout gestation and is required for the maintenance of pregnancy, but that at term PR-A expression may be induced and contribute to the 'functional' withdrawal of progesterone through its effects on PR-B.

Acknowledgement

We are grateful to Dr Patrick Moriarty (St Thomas's Hospital, London, UK) who provided the primary myocyte cultures.

References

Challis, J.R., Matthews, S.G., Van Meir, C. *et al*. (1995) Current topic: the placental corticotrophin-releasing hormone-adrenocorticotrophin axis. *Placenta*, **16**, 481–502.

Clarke, C.L., Zaino, R.J., Feil, P.D. *et al*. (1987) Monoclonal antibodies to human progesterone receptor: characterization by biochemical and immunohistochemical techniques. *Endocrinology*, **121**, 1123–1132.

Csapo, A. and Pinto-Dantas, C. (1965) The effect of progesterone on the human uterus. *Proc. Natl Acad. Sci. USA*, **54**, 1069–1076.

Cudeville, C., Mondon, F., Robert, B. *et al*. (2000) Evidence for progesterone receptors in the human fetoplacental vascular tree. *Biol. Reprod*., **62**, 759–765.

Haluska, G.J., Kaler, C.A., Cook, M.J. *et al*. (1994) Prostaglandin production during spontaneous labor and after treatment with RU486 in pregnant rhesus macaques. *Biol. Reprod*., **51**, 760–765.

Hovland, A.R., Powell, R.L., Takimoto, G.S. *et al*. (1998) An N-terminal inhibitory function, IF, suppresses transcription by the A-isoform but not the B-isoform of human progesterone receptors. *J. Biol. Chem*., **273**, 5455–5460.

How, H., Huang, Z.H., Zuo, J. *et al*. (1995) Myometrial estradiol and progesterone receptor changes in preterm and term pregnancies. *Obstet. Gynecol*., **86**, 936–940.

Karalis, K., Goodwin, G. and Majzoub, J.A. (1996) Cortisol blockade of progesterone: a possible molecular mechanism involved in the initiation of human labor. *Nat. Med.*, **2**, 556–560.

Kastner, P., Krust, A., Turcotte, B. *et al.* (1990) Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *EMBO J.*, **9**, 1603–1614.

Khan-Dawood, F.S. and Dawood, M.Y. (1984) Estrogen and progesterone receptor and hormone levels in human myometrium and placenta in term pregnancy. *Am. J. Obstet. Gynecol.*, **150**, 501–505.

Lye, S. (1994) The initiation and inhibition of labour—toward a molecular understanding. *Semin. Reprod. Endocrinol.*, **12**, 284–294.

McGarrigle, H.H. and Lachelin, G.C. (1984) Increasing saliva (free) oestriol to progesterone ratio in late pregnancy: a role for oestriol in initiating spontaneous labour in man? *Br. Med. J.* (*Clin. Res. Ed.*), **289**, 457–459.

Mohamed, M.K., Tung, L., Takimoto, G.S. *et al.* (1994) The leucine zippers of c-fos and c-jun for progesterone receptor dimerization: A-dominance in the A/B heterodimer. *J. Steroid Biochem. Mol. Biol.*, **51**, 241–250.

Padayachi, T., Pegoraro, R.J., Rom, L. *et al.* (1990) Enzyme immunoassay of oestrogen and progesterone receptors in uterine and intrauterine tissue during human pregnancy and labour. *J. Steroid Biochem. Mol. Biol.*, **37**, 509–511.

Rezapour, M., Backstrom, T., Lindblom, B. *et al.* (1997) Sex steroid receptors and human parturition. *Obstet. Gynecol.*, **89**, 918–924.

Sartorius, C.A., Melville, M.Y., Hovland, A.R. *et al.* (1994) A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isoform. *Mol. Endocrinol.*, **8**, 1347–1360.

Vegeto, E., Shahbaz, M.M., Wen, D.X. *et al.* (1993) Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. *Mol. Endocrinol.*, **7**, 1244–1255.

Westphal, U., Stroupe, S.D. and Cheng, S.L. (1977) Progesterone binding to serum proteins. *Ann. NY Acad. Sci.*, **286**, 10–28.

Received on January 18, 2001; accepted on June 18, 2001