Tissue expression of the nuclear progesterone receptor in male non-human primates and men

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Abstract

In females, progesterone is associated with reproductive functions. In males, its role and the expression of its genomic receptor are not very well understood. In attempts to achieve a hormonal male contraceptive method, gestagens are used to downregulate gonadotropin and sperm production. It is therefore essential to understand the mechanism of action of progesterone at the molecular level in males, especially in primates. This investigation was undertaken: (a) to determine whether the genomic progesterone receptor is expressed in males; and (b) to locate it in various organs that are potential targets of gestagens. Human tissues were obtained at surgery for benign prostatic hyperplasia or prostate cancer and at autopsy. Non-human primate tissues were obtained at autopsy. This study was performed by analyzing the

genomic progesterone receptor by immunohistochemistry, Western blot and RT-PCR. The nuclear progesterone receptor was expressed in pituitary and hypothalamus of both monkeys and men. In the testis progesterone receptor expression was found in a few peritubular and interstitial cells, but not in germ cells. In addition, expression was detected in the epididymis, prostate and male mammary gland. Reverse transcriptase (RT)-PCR experiments indicated that progesterone receptor A and B are expressed in all tissues analyzed. These data exclude direct genomic effects of gestagens at the spermatogenic level but indicate that a male contraceptive based on gestagens might have some effects on other tissues, such as the epididymis, prostate and mammary gland.

Journal of Endocrinology (2006) **189**, 529–539

Introduction

In males, the expression of the genomic progesterone receptor (PR) and the physiological function of progesterone at the molecular level are not well understood (Oettel & Mukhopadhyay 2004). Recently a non-genomic PR has been described which can be found in the membrane of sperm from a wide range of vertebrates (Zhu *et al.* 2003). Well-known non-genomic actions of the PR include stimulating sperm acrosome reaction and fertilization and other functions (Baldi *et al.* 1995, Alexander *et al.* 1996). In females, target organs of progesterone are ovary, uterus and mammary gland. In these organs the genomic PR is expressed in two major isoforms, called PR-A and PR-B, which are the product of two different transcriptional start sites and differ by an N-terminal extension of about 165 amino acids in the PR-B (Conneely *et al.* 2001). The mature PR-A and PR-B proteins have a molecular mass of about 94 and

116 kDa respectively. Luteinizing hormone (LH) induces PR expression in granulosa cells. PR-A and PR-B have partly different functions: in general PR-A is a repressor of PR-B. PR-B is a strong activator of target genes. The relative expression of PR-A and PR-B changes in different tissues and determines the type of action of gestagens. A shorter (60 kDa) isoform, called PR-C, has also been described (Wei & Miner 1994). The PR-C is translated from an in-frame ATG codon at the C-terminus of exon 2 of the PR gene and comprises the second zinc finger of the DNA-binding domain, the hormone binding domain and sequences for dimerization and nuclear localization. The PR-C has been shown to be localized in the cytoplasm, to bind progesterone and to migrate to the nucleus but is not capable of DNA binding. However, the PR-C can dimerize with the other isoforms, thereby modulating progesterone action by increasing the transcriptional activity of PR-A and PR-B (Wei *et al.* 1996).

Besides acting as a steroidogenic precursor and stimulating acrosome reaction (reviewed by Lösel *et al.* 2003), progesterone has no known major function in the male, and progesterone receptor-knock out (PRKO) male mice are phenotypically normal and fertile (Lydon *et al.* 1995). However, such male mice have elevated LH concentrations, suggesting a function of progesterone in the control of LH secretion (Schneider *et al.* 1999), an effect exploited in hormonal male contraception (Kamischke & Nieschlag 2004). In the current approach, in addition to testosterone, hormonal male contraceptives contain a gestagen (Nieschlag *et al.* 2003, Kamischke & Nieschlag 2004) which is exploited for gonadotropin suppressive properties. The feedback regulation of the hypothalamo-pituitary-gonadal axis by gestagens involves both inhibition of pulsatile gonadotropin-releasing hormone (GnRH) secretion and repression of GnRH receptor transcription via a progesterone responsive element present in the GnRH receptor promoter (Cheng *et al.* 2001, An *et al.* 2004).

It is not currently known which type of PR mediates progesterone and gestagen action in the male at the hypothalamo-pituitary level and in other organs. It was postulated that gestagen administered to men for contraceptive purposes might have a direct testicular and/or epididymal effect on spermatogenesis and sperm maturation (Kamischke *et al.* 2000a). Data on localization of the nuclear PR in the testis and other male organs, however, are scant. Dube and Tremblay (1979), found the PR in the testes of chickens and in patients with testicular feminization. In 1995, by reverse transcriptase (RT)-PCR, PR was found to be very highly expressed in the testis as well as the prostate of cynomolgus monkeys (Heikinheimo *et al.* 1995); however, no cellular localization study was performed. Very recently, strong expression of nuclear PR in the human seminiferous epithelium and testis was claimed (Shah *et al.* 2005). However, a careful review of the data presented in the human testis suggests the possibility of immunohistochemical staining artefacts and unspecific Western blot bands (Shah *et al.* 2005). In addition, we recently failed to demonstrate a direct biological testicular effect of norethisterone enanthate on the testis (Junaidi *et al.* 2005).

Characterizing the expression pattern of genomic PR in the male is necessary to design drugs acting selectively to suppress gonadotropins and spermatogenesis while avoiding side effects. In this study we analyzed nuclear PR expression in various reproductive and non-reproductive organs obtained from men and, as experimental models, from two non-human primate species, the marmoset and the cynomolgus monkey.

Materials and Methods

Tissues

Formaldehyde-fixed (4%), paraffin-embedded, male human tissues (liver, kidney, lung, spleen, heart, duodenum,

bladder, mammary gland, seminal vesicle, epidermis, pituitary and hypothalamus, $n=3$) and human ovary and placenta were archived material of the Institute of Pathology and of the Institute of Neuropathology, University Hospitals, Münster, Germany. Fresh human testes and epididymides were obtained at surgery from patients orchidectomized because of prostate cancer. These patients may have had an antiandrogen treatment prior to the surgery but, irrespective of this, the histology results were similar. Fresh prostate tissue samples were obtained at surgery for benign prostatic hyperplasia from three patients at the Urology Clinic, University Hospitals, Münster, Germany. Tissues were fixed in Bouin's solution for 4 h or snap frozen. Informed consent for the use of leftover tissues was provided by the patients, as required by the Ethics Committee of the Medical Faculty and State Medical Board. All tissues from both monkey species (cynomolgus, *Macaca fascicularis*; marmoset, *Callithrix jacchus*) were obtained at autopsy of male animals, euthanized for reasons independent from the present study, from our primate colony (licensed by the State Board according to the German Law for Animal Care and Protection). The monkey tissue samples were fixed in Bouin's solution for 4 h or snap frozen immediately after dissection. All chemically fixed tissues were embedded in paraffin, sectioned at 4 µm and used for immunohistochemistry. Snap-frozen tissues were kept at -80°C until use for Western blotting or RNA extraction. The human breast cancer cell line T47D was used as the positive control in all experiments.

Immunohistochemistry

The following tissues were tested immunohistochemically for the presence of the genomic PR: liver, kidney, lung, spleen, heart, duodenum, bladder, mammary gland, testis, epididymis, prostate, seminal vesicle, epidermis, pituitary and hypothalamus. All sections were treated equally. After deparaffinization and rehydration five different commercially available primary antibodies (Ab) for the human PR (four monoclonal, one polyclonal) were applied: mouse anti-PR Ab-3 (catalog number MS-193-P0) and mouse anti-PR Ab-7 (catalog number MS-197-P0) from Neomarkers, Fremont, CA, USA; mouse anti-PR (catalog number 2145) from Immunotech, Marseille, France; mouse anti-PR (catalog number NP008) from DAKO Diagnostika, Hamburg, Germany; rabbit anti-PR (catalog number A0098) also from DAKO Diagnostika. Antibodies were applied for 30 min at room temperature in blocking buffer. After washing, DAKO-LSAB 2 System (DAKO Diagnostika) was added for 30 min, followed by a washing step and by incubation with diaminobenzidine (DAB; DAKO Diagnostika) for 20 min. Controls for all immunohistochemical staining procedures were performed by omitting the primary antibody on adjacent

Figure 1 Schematic representation of the genomic organization of the PR gene covering exons 1 to 4. The exon sizes (nucleotides) are given in the different exon boxes. The two different primers are named B and AB and the transcript sizes are given below the primers. The starting and ending points (nucleotide position in the different exons) are given in the exon boxes. The primer pair for primer AB spans exon 3 and 4.

sections. The sections were counterstained with hematoxylin for 10 s and mounted under cover slips with Dako Faramount (DAKO Diagnostika) prior to observation using an upright microscope (Axioskop, Zeiss, Oberkochen, Germany) at different magnifications (objectives $\times 25$ and $\times 40$). Digital images of equal exposure were acquired with a CCD camera (Axiocam) controlled by image software (Axiovision).

Western blotting

Protein concentrations in cell homogenates in RIPA buffer were determined by BCA-protein assay (reagents from Pierce, Rockford, IL, USA). For electrophoresis NuPage pre-cast gels (Invitrogen) were run at 200 V and transferred at 30 V for 1 h to Hybond ECL-membranes (Amersham). After blocking with 5% milk powder in $1 \times$ PBS/0.01% Tween 20 blots were incubated overnight with primary anti-PR antibodies, washed and incubated with the appropriate secondary, horseradish peroxidase (HRP)-conjugated Ab (Sigma; A9044/1:30000) for 1 h. The mouse anti-PR Ab-3 (catalog number MS-193-P0) and anti-PR Ab-7 (catalog number MS-197-P0) from Neomarkers, and the mouse anti-PR (catalog number NP008) and rabbit anti-PR (catalog number A0098) from DAKO Diagnostika, were applied in Western blots, with similar results. As positive controls, a lysate from the breast cancer T47D cell line, obtained from CLS Heidelberg, Germany, and protein extracts from human and cynomolgus endometrium were used. Enhanced chemiluminescent detection was performed using the ECL plus kit (Amersham). In five repeated experiments, 10–20 μ g T47D cell lysates and various concentrations of tissue protein extracts (up to 800 μ g) were used.

RT-PCR

RNA was extracted from tissue stored at -80°C using a commercial kit (Ultraspec, Biotecx Laboratories, Houston, TX, USA). In addition, we used commercially available mRNA from human testis, epididymis and prostate (Biocat, Heidelberg, Germany). cDNA synthesis was performed using random primer examers. First-strand cDNA was synthesized starting from 2 μ g total RNA, 1 μ l random primers (Promega) and 12.5 μ l diethyl pyrocarbonate (DEPC) H_2O for 5 min at 65°C . Master mix (7 μ l) (containing 2 μ l dNTPs, 4 μ l M-MLV RT $5 \times$ buffer, 1 μ l RNasin and 0.5 μ l reverse transcriptase; all reagents from Promega) was added to a final volume of 20 μ l and the reaction was completed for 1 h at 37°C . Reactions were then frozen until use.

Amplification of PR-B was performed using the following primers in exon 1: PR-B forward primer, 5'-CCT GAAGTTTCGGCCATACCT-3'; PR-B reverse primer, 5'-AGCAGTCCGCTGTCCTTTTCT-3'. These primers were selected in a region of exon 1 presenting about 75% sequence identity between human, rat and mouse PR and produce an amplicon of 197 bp (Fig. 1). No marmoset sequence information is available in this genomic region.

Amplification of PR-A+B was performed amplifying a region encompassing parts of exon 3 and 4 using the following primers: PR-A+B forward primer, 5'-AGCA TGTCGCCTTAGAAAGTGC-3'; PR-A+B reverse primer, 5'-TAGGGCTTGCTTTTCATTTC-3'. The forward primer is located in exon 3 and presents 99% identity between human, mouse and rat sequences; the reverse primer, in exon 4, is 100% specific for both human and marmoset PR (marmoset accession no. Z86038).

This primer combination results in an amplicon of 148 bp.

Actin mRNA was amplified using the forward primer 5'-GATGACCCAGATCATGTTTG-3' and the reverse primer 5'-GAGCAATGATCTTGATCTTC-3'.

PR-B and PR-A+B were amplified in a duplex PCR reaction, and actin in a separate simplex PCR reaction. Reactions were performed in a 25 µl volume containing 2.5 µl 10 × PCR buffer with 15 mM MgCl₂ (Quiagen), 1 µl dNTPs (Promega), 1 µl of each primer, 5 µl 5 × Q solution (Quiagen), 0.3 µl Taq polymerase (5 IU/µl; Quiagen), 2 µl cDNA and 8.2 µl H₂O. PCR was performed in a RoboCycler (Stratagene, Amsterdam, The Netherlands). After initial denaturation (94 °C, 2 min) the 30 cycle amplification profile consisted of denaturation at 94 °C for 50 s, annealing at 58 °C for 40 s, and extension at 72 °C for 1 min. After a final extension at 72 °C for 10 min and cooling at 6 °C for 10 min, the amplified DNA products were resolved on a 1.8–2.0% agarose gel and visualized by ethidium bromide staining. Negative controls without RNA and without reverse transcriptase were also included in the PCR reaction to test for exogenous DNA contamination. To assure that the PCR-amplified DNA observed in the agarose gel represented the gene-specific product of interest, bands of the expected size were isolated (for the human tissues marker number VIII (Roche) and for the non-human primates testes marker 100 bp ladder (Peglab, Erlangen, Germany)), extracted, purified, cloned and sequenced.

Semi-quantification of the RT-PCR results was performed by image analysis in a MultiImage light cabinet (Biozym, Oldesdorf, Germany). Total fluorescent peak areas were calculated by ChemiImages software (Biozym). All data are expressed as mean ± S.D

Results

Immunohistochemistry

PR expression was first analyzed in all tissues (n=3 specimens each tissue) available from the three species by immunohistochemistry. The results are summarized in Table 1. PR was weakly but consistently expressed in the testis from human, cynomolgus monkey and marmoset (Fig. 2). All three species showed a similar expression pattern within the testis, whereby only very few peritubular and interstitial cell nuclei were positive. In the cynomolgus monkey (Fig. 2c and d), relatively more peritubular cell nuclei of the testis expressed the receptor compared with the human (Fig. 2a and b) and marmoset testis (Fig. 2e and f). In all species the staining was completely confined to the nuclei. No specific staining was ever observed in the seminiferous epithelium with any of the antibodies tested.

The prostate showed the strongest PR staining of all male tissues tested (Table 1). In the human and in

Table 1 PR expression analyzed by immunohistochemistry in human organs and in two different male non-human primate species

	Human	Cynomolgus	Marmoset
Organ			
Testis	+	+	+
Epididymis	+	++	+
Muscle	—	—	—
Pituitary	++	++	++
Hypothalamus	+	+	+
Prostate	++	+++	+++
Mammary gland	++	+	++
Placenta	+++	+++	+++
Ovary	++++	++++	++++

The level of expression is indexed by the number of plus characters assigned by the same observer by visual inspection compared with the control tissues (placenta and ovary).

cynomolgus monkey prostate staining was confined to the muscular layer of the stroma, showing strong signal in the nuclei of the smooth muscle cells below the epithelium (Fig. 3a and d). None of the other stromal cells showed staining.

In the epididymis, the PR was found in the peritubular cell nuclei of the monkeys (Fig. 3b for marmoset and Fig. 3e for cynomolgus monkey). Only in the cynomolgus epididymis were cells from the epidermal layer also positive for the signal (Fig. 3e). Apical cells occasionally showed a faint signal (not shown).

In the mammary gland, staining was confined to the epithelial layer of male breast ducts which were consistently strongly positive (Fig. 3g, cynomolgus monkey; Fig. 3h, marmoset). All immunohistochemical stainings were accompanied by positive control tissue for all three species, such as placenta or ovarian tissue (Fig. 3c, f and i).

In the hypothalamus and pituitary of the three species, sparse cells were consistently stained for the PR (Fig. 4). The other male organs investigated – i.e. liver, kidney, lung, spleen, heart, duodenum, bladder and epidermis – were negative.

Western blotting

Since the PR antibodies used for the immunohistochemical study do not clearly distinguish between the PR isoforms A, B and C, PR expression in selected tissues was further analyzed by Western blotting using the same antibodies as those for immunohistochemistry staining (Fig. 5). Repeated Western blot experiments showed that PR expression is probably too low to allow detection by this method in male tissue extracts. We analyzed: (a) testes from eight different men, from five different cynomolgus monkeys and nine different marmosets; (b) epididymidis from two different men, from two different cynomolgus monkeys and from two different marmosets; (c) prostate

Figure 2 Immunohistochemical expression pattern of the PR in sectioned testes biopsies with complete spermatogenesis at different magnifications counterstained with hematoxylin from men (a and b), cynomolgus monkeys (c and d) and marmosets (e and f). The localization of the PR for all tested species was similar. In all three species the PR expression was weak and exclusively located in the nucleus of some interstitial cells (double-tipped arrow) and in peritubular cells (single-tipped arrow). None of the tubular cells (germ or Sertoli cells) showed any staining for the receptor. Scale bars=50 µm.

from three different men with benign prostate hyoplasia (BPH), from two cynomolgus monkeys and from two marmosets. Even using very large amounts of proteins (up to 800 µg) and with all antibodies tested, no band corresponding to PR-A and PR-B could be detected in any tissue in the presence of the positive control (T47D cell extract, endometrium). A faint band, migrating just below the PR-C isoform was detected in the human prostate and in the marmoset epididymis (Fig. 5). However, given the

absence of PR-A protein in these tissues, this band might represent a non-specific product.

RT-PCR

Expression analysis by RT-PCR was performed using primers designed to detect only PR-B and PR-A+B in prostate, testis and epididymis. We analyzed: (a) testes

Figure 3 Immunohistochemical localization of the PR in male tissues (left and middle panels) and as control in female tissues (right panels) of the three tested species: human (a and c), cynomolgus monkey (d–g) and marmoset (b, h and i). The positive nuclei are stained with Newfuchsin and all sections are counterstained with hematoxylin. Smooth muscle cell nuclei of the prostate in men and cynomolgus monkeys are positive for the PR (a and d) as well as peritubular cell nuclei of the epididymis in marmosets and cynomolgus monkeys, but also some epithelial cells in the cynomolgus epithelial layer (b and e). In the male mammary gland the staining was confined to the epidermal tissue of male breast ducts (g for male cynomolgus monkey and h for male marmosets). All immunohistochemical staining was accompanied by positive control tissue for all three species, such as human placenta (c), ovarian tissue (f) and marmoset placenta (i). Scale bar = 100 μ m.

from four different men, from three different cynomolgus monkeys and three different marmosets; (b) epididymides from two men, from three cynomolgus monkeys and

from three marmosets; (c) prostate from three men with BPH, from three cynomolgus monkeys and from three marmosets. The PR expression was ubiquitous, although

Figure 4 Immunohistochemical expression pattern of the PR of hypothalamus (left panels) and pituitary (right panels) counterstained with hematoxylin from men (a and b), cynomolgus monkeys (c and d) and marmosets (e and f). The localization of the PR for all tested species was similar. In all three species the PR was located in scattered cell nuclei of the hypothalamus (arrow) and in nuclei of epithelial cells of the adenohypophysis (half-open arrow). Scale bars = 100 μ m.

with bands of different intensity, suggesting tissue-specific differences. mRNA expression analysis showed the presence of the PR-B and PR-A+B transcripts in all male human tissues analyzed including the testis, prostate and epididymis (Fig. 6, upper left panel). For the other two primate species both primer sets amplified the mRNA signals in the cynomolgus testis whereby in the marmoset the PR-A+B transcript was very faint and the isoform PR-B was not detected (Fig. 6, bottom left panel). The lack

of PR-B amplification in marmoset testis might be due to insufficient specificity for the marmoset sequence, unknown in this region.

Using commercially available, highly purified mRNA, we performed a semi-quantitative expression analysis after normalization against the housekeeping actin gene. In human prostate, epididymis and testis mRNA we detected a transcription level of both PR isoforms which was approximately 0.5-fold of that in T47D cells, without

Figure 5 Western blot analysis demonstrates specificity of the PR antibody for different PR isoforms (A, B and C). Although different male tissues were tested – i.e. testis, epididymis and prostate from three different primate species – no specific band was found. Only in the human prostate and in the marmoset epididymis was a faint band of approximately 60 kDa found. T47D cells and cynomolgus endometrium were used as controls.

major differences between isoforms and among tissues (Fig. 6, upper right panel). By analyzing three independent samples of cynomolgus monkey and marmoset testis we detected a similar expression level of both isoforms in the cynomolgus monkey but only the PR-A+B transcripts in the marmoset testis, at an expression level similar to that found in the cynomolgus testis (Fig. 6, lower right panel).

RNA extraction from human pituitaries and hypothalamic collected at autopsy was not successful and the PR expression pattern in these tissues could not be determined.

Discussion

Our study identifies some tissues as potential targets for genomic action of progesterone in men. These tissues include the hypothalamus and the pituitary gland, where progesterone possibly exerts its suppressive effect on gonadotropin secretion, the mammary gland and some organs of the male genital tract – including the prostate and the epididymis – while the presence of the genomic PR in the testis was rather weak.

Gestagens administered for hormonal male contraception reinforce the gonadotropin suppressive activity of

testosterone, resulting in azoospermia. It was suggested that the pituitary-suppressive action of progesterone in males is mediated by the genomic PR but no studies until now localized the genomic PR in human male hypothalamus and/or pituitaries (Brady *et al.* 2003). In this study we show for the first time that the genomic PR is present in sparse nuclei in male hypothalamus and pituitaries from humans and non-human primates. Co-localization experiments of the PR with gonadotropin and/or GnRH immunoreactivity are technically very demanding when applied to human specimens and our attempts have not been successful so far. Similarly, we could not determine which PR isoform is expressed in these tissues by RT-PCR, due to rapid RNA degradation after death. Our data, however, show that the genomic PR expression is similar in hypothalamus and pituitaries of human and non-human primates, providing the basis for future experiments in monkeys. Characterizing the type of PR involved in gonadotropin suppression might be useful for the design of selective PR modulators for male contraception. Recent experiments with a human neuronal medulloblastoma cell line suggested that PR-A and PR-B have different functions in the progesterone-mediated regulation of GnRH receptor promoter activity (An *et al.* 2004). Intriguingly, in these cells progesterone stimulated GnRH mRNA transcription, showing the complexity of

Figure 6 RT-PCR and semi-quantification of the PR isoforms in human, marmoset and cynomolgus monkey tissues. Relative quantification of RT-PCR products was performed by image analysis after normalization against actin. Upper panels: commercially available human mRNA was analyzed along with the positive control T47D. The upper right panel reports relative quantification results of three independent experiments. Lower panels: mRNA was isolated from testes obtained from three different marmoset and cynomolgus monkeys. The PR-A+B transcript was detected in all samples analyzed and was the only transcript in the marmoset testes (left panel). Right panel: relative quantification against actin. Data are given as mean $\pm$ S.D.

PR involvement in the regulation of the human GnRH system (An *et al.* 2004).

In this study we characterized the PR mRNA and protein expression in male reproductive organs such as the testis, the epididymis and the prostate. Our data do not support a major role for progesterone in the regulation of

testicular function. Our findings are in agreement with earlier studies in male non-human primates and men (Dube & Tremblay 1979, Heikinheimo *et al.* 1995), which were able to locate PR mRNA in the testis and in parts of the epididymis whereby they did not distinguish among the PR isoforms. Our data suggest that both PR-B

and PR-A are expressed in human and cynomolgus monkey testis. According to our results, confirmed by using different antibodies and equally treated positive controls, some peritubular cells and some interstitial cells are the only source of PR protein expression, but the low number of positive cells and the absence of PR protein by Western blotting indicate that primate males have only a very low expression of PR, making a physiological role of progesterone in the testis unlikely.

This result is also consistent with the literature since no other published studies have been able to demonstrate the existence of the PR in the testis, with one exception (Shah *et al.* 2005). These authors suggested that PR is expressed both in the nuclei and the cytoplasm of male germ cells in a stage-specific manner. However, some methodological aspects should be considered in the interpretation of these results (Shah *et al.* 2005). First, using tissue collected from cadavers, *in situ* hybridization revealed mRNA in male germ cell nuclei but not in the cytoplasm, where it would be expected. Secondly, immunohistochemistry data showed possibly unspecific, cytoplasmic staining only on one side of the slides, probably due to fixation or staining artefacts. Thirdly, no positive controls were employed. Using Western blots, bands were seen by loading only 50 µg of proteins, while, in the presence of the adequate positive control we could only detect a faint, possibly non-specific band at about 60 kDa by overloading the gels (800 µg) in all three primate species tested using four different antibodies. We also trust that our immunohistochemical results are valid because the tissues showed no signs of degeneration due to fixation, embedding or staining. Our histology results (see Figs 3 to 5) are uniform and consistent among three species in which germ cells never showed any staining in the nuclei or the cytoplasm. The possibility that our fixation protocol was too slow, and that the mRNA as well as the protein of the PR was lost due to degeneration, can be excluded because we found the expected PR expression pattern in equally treated tissues of female primates. Therefore, we conclude that the PR is only marginally expressed in peritubular and interstitial cells of human and non-human primate testis.

Tissue culture experiments have demonstrated that smooth muscle cells are a major source of PR mRNA, independent of gender (Hodges *et al.* 1999). PR expression is stimulated by estrogens. Besides testicular peritubular cells, we found PR expression in smooth muscle cells of the epididymis and prostate. Here the PR could exert a function similar to its role in other smooth muscle tissue cells, i.e. inhibition of calcium-ATPase and regulation of calcium influx, thereby hindering cell contraction and fluid transport (Fomin *et al.* 1999, Toshima *et al.* 2000, Saner *et al.* 2003, Welter *et al.* 2003). The PR expression found in the fibromuscular cells of the prostate confirms earlier findings in another primate, the rhesus monkey, which was treated with testosterone (West *et al.* 1988) and in humans with prostate cancer (Wolf *et al.* 1985).

The peritubular cells of the testis and the epididymal smooth muscle cells are needed to keep a constant flow of tubular and epididymal fluid containing maturing spermatids and sperm. PR might be involved in slowing this movement down. However, in the testis the number of PR-positive cells is very low so that such an effect may be minor. It should be considered that, together with the adrenal gland, the testis is exposed to the highest progesterone concentrations of the body and that the scant PR expression observed could be just a marker of estrogenic activity. As a non-genomic effect, progesterone stimulates sperm hyperactivation and acrosome reaction (Calogero *et al.* 2000), an effect certainly unwanted in the epididymis. PR expression in the epididymis might alter the flow (Ergün *et al.* 1997) and could also prevent sperm from premature acrosome reaction. It has been shown that norethisterone enanthate when given with testosterone reduces sperm motility more strongly than testosterone alone (Kamischke *et al.* 2000a). This is possibly caused by the binding of the epididymal PR to progesterone and decreases its bioavailability for premature sperm activation.

In summary, we have characterized the expression of the genomic PR in a number of male tissues. Our data suggest that genomic actions of progesterone are probably exerted at the hypothalamic-pituitary level, while a direct testicular effect of gestagens is unlikely. Although non-genomic progesterone functions have been found many in vertebrates (Zhu *et al.* 2003), our recent data showing that norethisterone enanthate has no direct testicular effect in the cynomolgus monkey confirms that gestagens play a very minor role directly in the testis *in vivo* (Junaidi *et al.* 2005). In addition, genomic PR is expressed in other organs, where gestagens could affect function when administered for contraceptive purposes.

Acknowledgements

We thank J Salzig, M Kloth and R Sandhowe for technical assistance, Professor Dr J Gromoll for helping in the gene expression analysis, and S Nieschlag for language editing of the manuscript.

Funding

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received in final form 13 March 2006

Accepted 21 March 2006

Made available online as an Accepted Preprint

24 March 2006