Non-genomic progesterone receptors in the mammalian ovary: some unresolved issues

Tony Bramley
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In addition to their well-documented genomic effects, steroid hormones may also exert actions that are: (i) rapid, (ii) insensitive to inhibitors of transcription, (iii) mimicked by steroids coupled to cell membrane-impermeant molecules, and (iv) demonstrable in cells that do not express the classic genomic progesterone receptor (gPR). Such ‘non-genomic’ effects have been described for all the major classes of steroids (progesterone, oestrogens, androgens and corticoids), as well as for thyroid hormones, retinoids and vitamin D3. Rapid, membrane-mediated effects of progesterone have been studied most intensively in human spermatozoa and in the Xenopus oocyte. However, similar non-genomic actions of progesterone and other steroids have now been described in a wide variety of different tissues in many species. The first putative membrane steroid receptor to be cloned was that for the pig membrane progesterone receptor (mPR). Subsequently, similar genes were cloned from rats and cattle, and two related mPRs have been described in humans. Despite accumulating evidence for cell-surface membrane actions of steroids, a number of uncertainties remain as to the properties and identity of such ‘receptors’ and their cellular actions. Furthermore, some rapid steroid effects may be mediated through membrane-associated ‘classical’ steroid receptors, and steroid receptors may be capable of activating other signalling pathways non-classically. This review focuses on some of these unresolved issues, taking as its model the actions of progesterone in the mammalian ovary.

Some of the earliest studies into steroid hormone action clearly showed that steroids could bind to specific, high-affinity binding sites (or receptors) localized on surface-membrane fractions of their target cells (Pietras and Szego, 1977). However, other laboratories demonstrated the presence of specific steroid receptors in the nuclear–cytosolic fractions of target cells and showed that such receptors could act as ligand-dependent transcription factors capable of modulating the activity of specific genes that were known to be regulated by steroid hormones. These findings led, understandably, to the concentration of effort on elaborating the classical genomic steroid hormone actions. However, there has been a recent resurgence of interest in the actions of steroids at the cell surface membrane, and a growing realization that some effects of steroid hormones clearly cannot be explained by a change in transcription (Table 1). A number of excellent reviews have appeared in the last few years that discuss in detail non-genomic effects of progesterone (Sutter-Dub, 2002) and other steroids (Wehling, 1997; Revelli et al., 1998; Falkenstein et al., 2000a; Schmidt et al., 2000; Kelly and Levine, 2001).

Progesterone is an important intermediate in the synthesis of androgens and oestrogens. It plays an important role in ovulation, atresia and luteinization in vivo (Telleria and Deis, 1994; Chaffin and Stouffer, 2000), and is essential for the continuation of early pregnancy in all mammalian species. However, the importance of genomic and non-genomic responses to progesterone in the mammalian ovary is still being debated. Some of the issues addressed in this review are:

- Which responses to progesterone are mediated through classical genomic effects, non-classical genomic or non-genomic actions?
- Which regions of the receptor protein or gene are detected by different antibodies or primers?
- What isoforms or variants of the genomic or membrane receptor(s) are expressed in progesterone target cells and in what proportions?
- How do cross-talk with other hormone and growth factor signalling processes, post-translational receptor

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- Which regions of the receptor protein or gene are detected by different antibodies or primers?
- What isoforms or variants of the genomic or membrane receptor(s) are expressed in progesterone target cells and in what proportions?
- How do cross-talk with other hormone and growth factor signalling processes, post-translational receptor
modification, and type of cell influence the cellular response to progesterone?

### Classical genomic progesterone receptors

The genomic progesterone receptor is a member of the ligand-inducible DNA-binding superfamily of nuclear transcription factors that includes the receptors for the other steroid hormones, thyroid hormones, vitamin D, retinoic acid and a variety of ‘orphan’ receptors. The cDNA for the B-form of the (human) genomic retinoic acid and a variety of ‘orphan’ receptors.

**Classical genomic progesterone receptors**

The genomic progesterone receptor is a member of the ligand-inducible DNA-binding superfamily of nuclear transcription factors that includes the receptors for the other steroid hormones, thyroid hormones, vitamin D, retinoic acid and a variety of ‘orphan’ receptors. The cDNA for the B-form of the (human) genomic progesterone receptor (gPR-B) consists of eight different exons separated by seven short introns, and codes for a 933 amino acid (116 kDa) protein containing a number of different functional domains (Fig. 1). Exon 1 codes for the N-terminal region of the receptor and for one of three activation function domains (AF-3). Exons 2 and 3 code for AF-1 and the DNA-binding domain (DBD) with its two zinc fingers. Exons 4–8 code for the ligand-binding domain (LBD) and AF-2. This region also codes for the hinge region, a nuclear localization signal (NLS), and regions important for receptor dimerization and binding of heat-shock proteins and immunophilins. Two other isoforms of the human gPR arise from the use of alternate promoters within the same gene. PR-A is N-terminally truncated (94 kDa), lacking 164 amino acids containing the AF-3 domain (Giangrande and McDonnell, 1999), whereas PR-C (60 kDa) lacks exon 1 and most of exon 2 (Wei and Miner, 1994).

Several other variants of gPR have been described (Fig. 1). One variant codes for a protein that lacks the NLS, hinge region and part of the LBD; another variant has a frameshift that gives rise to a truncated protein lacking much of the LBD (Hodges et al., 1999). In addition, other PR variants have been described that have deletions of one or more exons (Misao et al., 1998a; 2000) or that have previously unidentified 5’ sequences spliced to novel exons, yet still retain those exons (exons 4–8) that code for the LBD (Hirata et al., 2000, 2002). Variants lacking the LBD or DBD can modify the effects of full-length wild-type gPR-B (Vegeto et al., 1993; Wei et al., 1997; Hodges et al., 1999; Misao et al., 2000).

### Effects of progesterone and anti-gestagens on ovarian function: classical genomic, non-classical genomic or non-genomic effects?

Some of the known responses of ovarian tissues to progesterone in vitro are listed for domestic animals (Table 2), humans and primates (Table 3) and rats (Table 4). Both the dose–response characteristics and sensitivity to anti-progestins of rapid and longer-term responses are distinct. Stimulation of intracellular Ca2+ and inositol trisphosphate (IP3) (and protein kinase C B1 (PKC-B1)) by progesterone is detectable within seconds at picomolar levels and is not blocked by anti-gestagens. In general, longer-term actions (effects on steroidogenesis, apoptosis and proliferation; activation of protein or mRNA synthesis) are detectable only at much higher (nanomolar to micromolar) concentrations of progesterone, and are reversed by anti-gestagens (Tables 2–4), implying action via classical genomic progesterone receptors. To date, rapid Ca2+, IP3, phospholipase C-β (PLC-β) responses have been studied only in pig granulosa cells, although similar rapid and specific responses to androgen and oestrogen have also been observed in pig granulosa cells (Lieberherr et al., 1999) and to other steroids in cells lacking classical steroid receptors (Le Mellay et al., 1999). Whether rapid response to very low progesterone concentrations is a general response in the ovarian cells of other mammalian species is still unclear.

The actions of progesterone and synthetic progestagens may differ (for review, see Swan et al., 2002). Indeed, although [3H]progesterone tracer bound readily to plasma-membrane binding sites in the bovine ovary (Rae et al., 1998), 3β-glucuronide-11-125I-labelled tyramine–progesterone and progesterone coupled to radiolabelled BSA were poor ligands (Bramley et al., 2002). Moreover, a number of synthetic progestagen agonists (levonorgestrel, medroxyprogesterone acetate, ORG2058, R5020) and antagonists (mifepristone, onapristone) with high activity for classical gPRs failed to compete for ovarian [3H]progesterone binding sites (Rae et al., 1998), indicating that progestagenic agonists, derivatives and antagonist analogues may not behave in the same manner as progesterone itself.

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**Table 1. Comparison of some characteristics of genomic and non-genomic effects of steroid hormones**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Genomic effect</th>
<th>Non-genomic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency of action?</td>
<td>Slow (normally several hours)</td>
<td>Rapid (seconds to minutes)</td>
</tr>
<tr>
<td>Inhibitors of transcription?</td>
<td>Effect blocked</td>
<td>Effect persists</td>
</tr>
<tr>
<td>Cells lacking a nucleus?</td>
<td>No effect demonstrable</td>
<td>Effect persists</td>
</tr>
<tr>
<td>Cells lack genomic receptor?</td>
<td>No effect demonstrable</td>
<td>Effect persists</td>
</tr>
<tr>
<td>Isolated cell membranes?</td>
<td>No effect demonstrable</td>
<td>Effects demonstrable</td>
</tr>
<tr>
<td>Cell-impermeable steroid?</td>
<td>No effect demonstrable</td>
<td>Effect persists</td>
</tr>
<tr>
<td>Inhibitors of ‘classic’ genomic steroid receptor?</td>
<td>Effect blocked</td>
<td>Effect persists</td>
</tr>
</tbody>
</table>

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**Table 2. Effects of progesterone and anti-gestagens on ovarian function: classical genomic, non-classical genomic or non-genomic effects?**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Genomic effect</th>
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<tr>
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<td>Effects demonstrable</td>
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<tr>
<td>Cell-impermeable steroid?</td>
<td>No effect</td>
<td>Effect persists</td>
</tr>
<tr>
<td>Inhibitors of ‘classic’ genomic steroid receptor?</td>
<td>Effect blocked</td>
<td>Effect persists</td>
</tr>
</tbody>
</table>

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**Table 3. Inhibitors of ‘classic’ genomic progesterone receptor (gPR) actions**

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Effect blocked</th>
<th>Effect persists</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat-shock proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunophilins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two other PR variants</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Table 4. Effects of progesterone and anti-gestagens on ovarian function: classical genomic, non-classical genomic or non-genomic effects?**

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<th>Condition</th>
<th>Genomic effect</th>
<th>Non-genomic effect</th>
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<tbody>
<tr>
<td>Latency of action?</td>
<td>Slow</td>
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</tr>
<tr>
<td>Inhibitors of transcription?</td>
<td>Effect blocked</td>
<td>Effect persists</td>
</tr>
<tr>
<td>Cells lacking a nucleus?</td>
<td>No effect</td>
<td>Effect persists</td>
</tr>
<tr>
<td>Cells lack genomic receptor?</td>
<td>No effect</td>
<td>Effect persists</td>
</tr>
<tr>
<td>Isolated cell membranes?</td>
<td>No effect</td>
<td>Effects demonstrable</td>
</tr>
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<td>Effect blocked</td>
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</tbody>
</table>

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**Table 5. Characteristics of genomic and non-genomic effects of steroid hormones**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Genomic effect</th>
<th>Non-genomic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibitors of transcription?</td>
<td>Effect blocked</td>
<td>Effect persists</td>
</tr>
<tr>
<td>Cells lacking a nucleus?</td>
<td>No effect</td>
<td>Effect persists</td>
</tr>
<tr>
<td>Cells lack genomic receptor?</td>
<td>No effect</td>
<td>Effect persists</td>
</tr>
<tr>
<td>Isolated cell membranes?</td>
<td>No effect</td>
<td>Effects demonstrable</td>
</tr>
<tr>
<td>Cell-impermeable steroid?</td>
<td>No effect</td>
<td>Effect persists</td>
</tr>
<tr>
<td>Inhibitors of ‘classic’ genomic steroid receptor?</td>
<td>Effect blocked</td>
<td>Effect persists</td>
</tr>
</tbody>
</table>
Ovarian genomic progesterone receptors

The presence and cellular localization of gPR proteins and mRNA in ovarian tissues has been demonstrated by immunocytochemistry and in situ hybridization (Tables 5 and 6). Most of these studies have used antibodies and primers designed to detect either the ligand binding domain (LBD) of the gPR, or the N-terminus (to differentiate PR-A and PR-B isoforms). PR-A and PR-B have been localized simultaneously in the same types of cell (Mote et al., 1999). It will be of interest to examine the distribution of PR-A and PR-B isoforms in types of ovarian cell at different stages of the ovarian cycle, and to study their responses to luteotrophic and luteolytic stimuli.

The co-expression of PR-A or PR-C in the same type of cell as PR-B modulates its activity (Vegeto et al., 1993; Wei et al., 1997). Moreover, genomic actions of progesterone receptor isoforms are affected differentially by their association with chaperones (Smith, 2000) and nuclear coactivators (Rowan and O’Malley, 2000), and are modulated by receptor phosphorylation (Orti et al., 1992) and by cross-talk with other signalling pathways (mitogen-activated protein kinase (MAPK), Src, tyrosine kinases, phosphatidyl inositol 3-kinase (PI3-K)) that are activated by growth factors and cytokines (Richer et al., 1998; Boonyaratanaoknit et al., 2001; Bagowski et al., 2001). Furthermore, gPRs can exist in different functional states (Smith et al., 2000), and can exhibit direct nongenomic actions in their unliganded (Weigel and Zhang, 1998) and undimerized states (Cohen-Solal et al., 1993). Finally, PR-A and PR-B isoforms can activate different constellations of genes even within the same cell (Richer et al., 2002), highlighting the enormous complexity of the intracellular processes involved in progesterone activation of a target cell.

Non-genomic membrane receptors for progesterone

Steroids can affect membrane-mediated events nongenomically in a number of ways (for example, by effects on membrane fluidity, or by direct effects on other G-protein-coupled receptors; for review, see Falkenstein et al., 2000b). However, studies of rapid, steroid-specific responses to progesterone, mediated by the cell membrane, culminated in the isolation and cloning of a non-genomic (pig) progesterone receptor of 194 amino acids, with a single membrane-spanning domain (Falkenstein et al., 1996; Meyer et al., 1996). Homologous proteins were subsequently cloned in rats (Selmin et al., 1996; Krebs et al., 2000; Nolte et al., 2000) and cattle (Cenedella et al., 1999; Zhu et al., 2001), and two putative mPR homologues (hpr6.6 and Dg6) were identified in humans (Gerdes et al., 1998). The human mPR gene consists of three exons separated by two introns. The 5′-region lacks a typical TATA box, but has high homology to a transcription initiator consensus sequence. The proximal region is GC-rich, and a CpG island spans the putative transcription start site (Bernauer et al., 2001). Several upstream regulatory DNA motifs were identified, including AP2, NF-AT, C/EBP and Ahr/Arnt (Bernauer et al., 2001).

Although the rapid effects of progesterone are mediated at the cell surface membrane of a variety of types of cell in pig hepatocytes (Falkenstein et al., 1998) and in Chinese hamster ovary cells transfected with mPR (Falkenstein et al., 1999), progesterone binding is associated with the endo-membrane rather than the cell surface-membrane fraction. Nevertheless, the protein was exposed at the cell surface of human spermatozoa, as an antibody to the protein prevented induction of the acrosome reaction by progesterone, but not by other
### Table 2. Effects in vitro of progesterone on the ovary of domestic species

<table>
<thead>
<tr>
<th>Species</th>
<th>Type of cell</th>
<th>Effective dose of Progestagen</th>
<th>Effective dose of Antiprogestagen</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>Granulosa</td>
<td>1 μmol P l⁻¹</td>
<td>1 μmol RU486 l⁻¹</td>
<td>Increased [Ca²⁺], IP₃</td>
<td>Machelon et al., 1996*║</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 μmol P l⁻¹</td>
<td>1 μmol RU486 l⁻¹</td>
<td>Increased [Ca²⁺],IP₃, PKC-β1</td>
<td>Lieberherr et al., 1999*║</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 nmol RU486 l⁻¹</td>
<td>30 nmol ONA l⁻¹</td>
<td>Upregulated gPR and inhibited E₂/P accumulation</td>
<td>Wu et al., 1999</td>
</tr>
<tr>
<td></td>
<td>Stable granulosa cell line (JC-410)</td>
<td>3–10 μmol LNG l⁻¹</td>
<td>1–10 μmol RU486 l⁻¹</td>
<td>LNG and RU stimulated P accumulation. P, RU and LNG increased P450scc transcription. RWJ inhibited P450scc expression.</td>
<td>Swan et al., 2002‡</td>
</tr>
<tr>
<td></td>
<td>Theca</td>
<td></td>
<td>30 nmol RU486 l⁻¹</td>
<td>Inhibited P secretion</td>
<td>Wu et al., 1999</td>
</tr>
<tr>
<td>Sheep</td>
<td>Ovarian surface epithelium cells</td>
<td>3 nmol P l⁻¹</td>
<td></td>
<td>Upregulated p53 and inhibited E₂-stimulated proliferation</td>
<td>Murdoch and Van Kirk, 2002†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 nmol P l⁻¹</td>
<td>60 nmol RU486 l⁻¹</td>
<td>Increased PARP activity</td>
<td>Murdoch, 1998†, Murdoch et al., 2001†</td>
</tr>
<tr>
<td>Cow</td>
<td>Granulosa</td>
<td>1 μmol MPA l⁻¹</td>
<td>0.1 μmol RU486 l⁻¹</td>
<td>inhibited upregulation of oxytocin gene</td>
<td>Lioutas et al., 1997†</td>
</tr>
<tr>
<td></td>
<td>Cumulus–oocyte complex</td>
<td>10 μmol P l⁻¹</td>
<td>1 μmol ONA l⁻¹</td>
<td>Inhibited E₂ secretion</td>
<td>Mingoti et al., 2002</td>
</tr>
<tr>
<td></td>
<td>Corpus luteum</td>
<td>50 nmol P l⁻¹</td>
<td>50 nmol RU486 l⁻¹</td>
<td>P inhibited and RU and ONA stimulated apoptosis induced by AG</td>
<td>Rueda et al., 2000†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 μmol P l⁻¹</td>
<td>5 μmol ONA l⁻¹</td>
<td>Decreased PGF₂α and prostacyclin secretion</td>
<td>Pate, 1988</td>
</tr>
</tbody>
</table>

*Demonstration of cell surface receptor action.
†Progestin effect reversed by gPR antagonist.
‡Progestin effect not reversed by gPR antagonist.
§Antiprogestin effect reversed by progestin.

AG: aminoglutethimide; E₂: oestradiol; gPR: genomic progesterone receptor; IP₃: inositol trisphosphate; LNG: levonorgestrel; MPA: medroxyprogesterone acetate; ONA: onapristone (ZK98299); P: progesterone; P450scc: cytochrome P450 side-chain cleavage; PARP: pituitary adenyl cyclase activated regulatory protein; PKC-β1: protein kinase C β1; RU486: mifepristone; RWJ: RWJ26819.
Table 3. Effects *in vitro* of progesterone on the human ovary

<table>
<thead>
<tr>
<th>Type of cell</th>
<th>Effective dose range</th>
<th>Progestagen</th>
<th>Antiprogestagen</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulosa (luteinizing IVF cells)</td>
<td>1 nmol–10 μmol P I^{-1}</td>
<td>50 μmol RU486 I^{-1}</td>
<td>10 μmol ORG</td>
<td>Induced apoptosis and stimulated caspase-3</td>
<td>Svensson et al., 2001$^\S$</td>
</tr>
<tr>
<td></td>
<td>10 nmol P I^{-1}</td>
<td>1 μmol RU486 I^{-1}</td>
<td></td>
<td>P reduced apoptosis and had protective effect on survival</td>
<td>Makrigiannakis et al., 2000$^\T$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 μmol RU486 I^{-1}</td>
<td>50 μmol HRP I^{-1}</td>
<td>Reduced secretion of (E_2) P and relaxin</td>
<td>VanderVoort et al., 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5–10 nmol RU486 I^{-1}</td>
<td></td>
<td>Suppressed P secretion</td>
<td>DiMattina et al., 1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50–500 μmol RU486 I^{-1}</td>
<td></td>
<td>Inhibited 17-hydroxylase. No effect on E_2 secretion</td>
<td>DiMattina et al., 1987</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10–100 nmol RU486 I^{-1}</td>
<td></td>
<td>No effect on basal, hCG- or PGE_2-stimulated P</td>
<td>Greenberg et al., 1990</td>
</tr>
<tr>
<td></td>
<td></td>
<td>640 nmol P I^{-1}</td>
<td>64 nmol RU486 I^{-1}</td>
<td>P inhibited proliferation and differentiation</td>
<td>Chaffkin et al., 1993$^\D$</td>
</tr>
<tr>
<td>Ovarian surface epithelium cells</td>
<td>160 nmol P I^{-1}</td>
<td>100 μmol ORG I^{-1}</td>
<td></td>
<td>Suppressed proliferation. Enhanced proliferation</td>
<td>Ivarsson et al., 2001$^\T$</td>
</tr>
<tr>
<td>Corpus luteum</td>
<td></td>
<td>10 nmol RU486 I^{-1}</td>
<td></td>
<td>Inhibited hCG-stimulated (but not basal) P secretion</td>
<td>Ottander et al., 2000</td>
</tr>
<tr>
<td>SKOV-3 ovarian cancer</td>
<td>30–300 nmol P I^{-1}</td>
<td>3 μmol RU486 I^{-1}</td>
<td></td>
<td>Inhibited uPA secretion and cell invasiveness</td>
<td>McDonnel and Murdoch, 2001$^*\D$</td>
</tr>
<tr>
<td>gPR-positive ovarian carcinoma</td>
<td>30–120 nmol RU486 I^{-1}</td>
<td></td>
<td></td>
<td>Inhibited proliferation (blocked G_s/G_1 phase of cell cycle) and downregulated gPR</td>
<td>Rose and Barnea, 1996</td>
</tr>
</tbody>
</table>

$^*\text{Demonstration of cell surface receptor action.}$  
$^\D\text{Progestin effect reversed by gPR antagonist.}$  
$^\S\text{Progestin effect not reversed by gPR antagonist.}$  
$^\T\text{Antiprogestin effect not reversed by progestin.}$

E_2: oestradiol; gPR: genomic progesterone receptor; HRP: horseradish peroxidase; ORG: ORG-5128; P: progesterone; PGE_2: prostaglandin E_2; RU486: mifepristone; SKOV-3: human ovarian cancer cell line; uPA: urokinase plasminogen activator.
Table 4. Effects in vitro of progesterone on the rat ovary

<table>
<thead>
<tr>
<th>Type of cell</th>
<th>Progestagen</th>
<th>Antiprogestagen</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulosa cells</td>
<td>1 µmol P l⁻¹</td>
<td>Enhanced LH- and FSH-stimulated steroidogenesis</td>
<td>Fanjul et al., 1983</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 µmol R5020 l⁻¹</td>
<td>Inhibited aromatase. Inhibited P secretion</td>
<td>Fortune and Vincent, 1983</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300 nmol RU486 l⁻¹</td>
<td>P reduced and antagonist enhanced apoptosis and cell adhesion</td>
<td>Peluso and Pappalardo, 1994</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 µmol RU486 l⁻¹</td>
<td>Antagonized hCG-induced apoptosis</td>
<td>Svensson et al., 2000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 µmol P l⁻¹</td>
<td>Inhibition of hCG- and forskolin-induced PACAP mRNA expression</td>
<td>Ko et al., 1999</td>
<td></td>
</tr>
<tr>
<td></td>
<td>64 µmol RU486 l⁻¹</td>
<td>Inhibited apoptosis and MAPKK</td>
<td>Peluso et al., 2001*</td>
<td></td>
</tr>
<tr>
<td>Large granulosa cells</td>
<td>640 nmol P l⁻¹</td>
<td>P inhibited apoptosis of large GC Stimulated bFGF secretion and inhibited apoptosis in large luteal cells</td>
<td>Luciano et al., 1994†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>64 nmol P l⁻¹</td>
<td></td>
<td>Peluso and Pappalardo, 1999*</td>
<td></td>
</tr>
<tr>
<td>Small granulosa cells</td>
<td>160 nmol P l⁻¹</td>
<td>P inhibited insulin-stimulated mitosis</td>
<td>Luciano and Peluso, 1995†</td>
<td></td>
</tr>
<tr>
<td>Granulosa/SIGC</td>
<td>640 nmol P l⁻¹</td>
<td>Inhibited apoptosis and MAPKK</td>
<td>Peluso et al., 2001*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>640 nmol RU486 l⁻¹</td>
<td>Induced GRE expression; RU486 and ONA antagonized P effect, but did not block LH-induction of gPR</td>
<td>Natraj and Richards, 1993†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>640 nmol ONA l⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteinizing granulosa cells</td>
<td>10 µmol ONA l⁻¹</td>
<td>Inhibited hCG and forskolin on PAC-1 receptor expression</td>
<td>Ko and Park-Sarge, 2000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>150 nmol P l⁻¹</td>
<td>Inhibited hCG and forskolin on PAC-1 receptor expression</td>
<td>Ko and Park-Sarge, 2000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 µmol ONA l⁻¹</td>
<td>Recombinant human PACAP mRNA expression</td>
<td>Natraj and Richards, 1993†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 µmol RU486 l⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL cells</td>
<td>10 µmol LNG l⁻¹</td>
<td>Inhibited LH and cAMP stimulated P secretion</td>
<td>Telleria and Deis, 1994</td>
<td></td>
</tr>
<tr>
<td>CL of pregnancy</td>
<td>0.1 µmol P l⁻¹</td>
<td>Suppressed IL-6 expression</td>
<td>Telleria et al., 1998</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1–100 µmol R5020 l⁻¹</td>
<td>Stressed P secretion</td>
<td>Telleria et al., 1999</td>
<td></td>
</tr>
<tr>
<td>CL and SV40 sensitive cell line</td>
<td>10 nmol P l⁻¹</td>
<td>Inhibited 20α-HSD expression</td>
<td>Sugino et al., 1997§</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 µmol RU486 l⁻¹</td>
<td>Inhibited superoxide production</td>
<td>Sugino et al., 1996†</td>
<td></td>
</tr>
<tr>
<td>CL-derived phagocytes</td>
<td>300 nmol P l⁻¹</td>
<td>Inhibited superoxide production</td>
<td>Sugino et al., 1996†</td>
<td></td>
</tr>
</tbody>
</table>

*Demonstration of cell surface receptor action.
†Progestin effect reversed by gPR antagonist.
‡Progestin effect not reversed by gPR antagonist.
§Effect may be explained by action at the glucocorticoid receptor.

bFGF: basic fibroblast growth factor; CL: corpus luteum; GC: granulosa cell; gPR: genomic progesterone receptor; GRE: glucocorticoid response element; 20α-HSD: hydroxysteroid dehydrogenase; IL-6: interleukin 6; LNG: levonorgestrel; MAPKK: mitogen-activated protein kinase kinase; ONA: onapristone (ZK98299); ORG: ORG-5128; P: progesterone; PACAP: pituitary adenylate cyclase activating polypeptide; PAC-1: PACAP-specific receptor-1; RU486: mifepristone.
agonists (Falkenstein et al., 1999; Buddhikot et al., 1999).

What is the nature of the membrane receptor for progesterone?

There is conflicting data on the nature of the membrane progesterone receptor, and the signalling pathways used in different types of cell (Fig. 2). Studies of rapid, membrane-mediated events in several types of cell have identified a wide range of changes, including Ca\(^{2+}\) mobilization (influx or mobilization from intracellular stores), opening of Na\(^+\) and Cl\(^{-}\) channels, and the activation of phospholipase C (leading to increased intracellular IP\(_3\) and diacylglycerol generation), Pertussis toxin-insensitive G-protein-coupled receptors, PKC, tyrosine kinase and MAPK pathways (for review, see Wehling, 1997; Falkenstein et al., 2000a). However, it is unclear which of these changes are primary, and which are activated by other downstream events or by cross-talk with other signalling pathways. Although the rat ovarian mPR has some similarity to gamma amino butyric acid A receptors (GABA\(_A\)) (Peluso and Pappalardo, 1998), GABA agonists and antagonists failed to affect progesterone binding to bovine luteal membranes (Menzies et al., 1999).

The two most widely used models for studies of the non-genomic progesterone receptor are human spermatozoa (which had been thought to lack the genomic PR; Castilla et al., 1995) and the Xenopus oocyte (in which responses in enucleated cells, and membrane-impermeable derivatives of progesterone can mimic all the rapid changes induced by the steroid). However, doubts about these models have been raised as (a) spermatozoa appear to contain small amounts of gPR mRNA (Sachdeva et al., 2000; Contreras and Llanos, 1999).
<table>
<thead>
<tr>
<th>Species</th>
<th>Primers (nucleotides)</th>
<th>Method</th>
<th>Type of cell</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>2113–2343</td>
<td>RT–PCR (PR-B)</td>
<td>CL</td>
<td>Ottander et al., 2000</td>
</tr>
<tr>
<td></td>
<td>144–367</td>
<td>RT–PCR (PR-A/B)</td>
<td>CL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Southern blots/ISH</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1341–1360</td>
<td>RT–PCR/Southern blot</td>
<td>CL</td>
<td>Misao et al., 1998a</td>
</tr>
<tr>
<td>PR-A and PR-B</td>
<td>1817–36 and 2330–49</td>
<td>Quantitative RT–PCR</td>
<td>CL</td>
<td>Misao et al., 1998b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Southern blot</td>
<td>HOSE cells</td>
<td>Lau et al., 1999</td>
</tr>
<tr>
<td>Monkey</td>
<td>LBD</td>
<td>RT–PCR, sequencing,</td>
<td>Germinal epithelium, CL,</td>
<td>Chandrasekher et al., 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>restriction enzyme</td>
<td>luteinizing GC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LBD</td>
<td>RPAs</td>
<td>CL, luteinized GC</td>
<td>Duffy et al., 1996</td>
</tr>
<tr>
<td></td>
<td>LBD</td>
<td>Northern blot, RPAs</td>
<td>CL</td>
<td>Duffy and Stouffer, 1995</td>
</tr>
<tr>
<td>Baboon</td>
<td>Full length hPR</td>
<td>Northern blot</td>
<td>CL</td>
<td>Hild-Petito and Fazleabas, 1997</td>
</tr>
<tr>
<td>Bovine</td>
<td></td>
<td>RPAs/northern blot</td>
<td>GC</td>
<td>Lioutas et al., 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RT–PCR, sequencing</td>
<td>CL</td>
<td>Rueda et al., 2000</td>
</tr>
<tr>
<td></td>
<td>Hinge/LBD</td>
<td>Northern blot</td>
<td>Periovulatory follicle</td>
<td>Cassar et al., 2002</td>
</tr>
<tr>
<td>Rat</td>
<td>LBD</td>
<td>RT–PCR, ISH</td>
<td>GC of preovulatory follicles</td>
<td>Park-Sarge et al., 1995</td>
</tr>
<tr>
<td></td>
<td>LBD</td>
<td>RT–PCR</td>
<td>Transient expression in</td>
<td>Park and Mayo, 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GC of luteinizing primed</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ovary</td>
<td></td>
</tr>
</tbody>
</table>

CL: corpus luteum; GC: granulosa cell; HOSE: human ovarian surface epithelium cells; hPR: human progesterone receptor; ISH: in situ hybridization; LBD: ligand-binding domain; PR-A/B: progesterone receptor-A or -B; RPAs: ribonuclease protection assay.
Table 6. Studies demonstrating genomic progesterone receptor protein expression in ovarian tissues

<table>
<thead>
<tr>
<th>Species</th>
<th>Antibody</th>
<th>Protein detected</th>
<th>Type of cell</th>
<th>Subcellular localization</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Range of mAbs</td>
<td>116 and 97 kDa</td>
<td>Ovarian tumours, CL, stroma, HOSE</td>
<td>Nuclear</td>
<td>Press and Green, 1988</td>
</tr>
<tr>
<td></td>
<td>mAb C262</td>
<td>120 and 90 kDa</td>
<td>CL</td>
<td>Nuclear</td>
<td>Iwai et al., 1990</td>
</tr>
<tr>
<td></td>
<td>mAb C262</td>
<td>116 and 90 kDa</td>
<td>GC of dominant follicles</td>
<td>Nuclear</td>
<td>Misao et al., 1998a</td>
</tr>
<tr>
<td></td>
<td>Neomarkers hPRA3</td>
<td>PR-B and PR-A</td>
<td>Ovarian cancers</td>
<td>Nuclear</td>
<td>Akahira et al., 2000</td>
</tr>
<tr>
<td></td>
<td>Neomarkers hPRA7</td>
<td>PR-A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neomarkers hPRA2</td>
<td>PR-B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Novocastra mAb</td>
<td>120 and 90 kDa</td>
<td>Theca and small and large luteal cells</td>
<td>Nuclear</td>
<td>Suzuki et al., 1994</td>
</tr>
<tr>
<td></td>
<td>JZB39 mAb</td>
<td>60–80% of luteinizing GC</td>
<td>Germinat epithelium, CL, cancer</td>
<td>Nuclear</td>
<td>Duffy et al., 1996</td>
</tr>
<tr>
<td></td>
<td>Abbott K68</td>
<td>60 kDa</td>
<td>Germinal epithelium, CL, GC of some follicles</td>
<td>Nuclear?</td>
<td>Hild-Petito and Fazleabas, 1997</td>
</tr>
<tr>
<td></td>
<td>Abbott</td>
<td>60 kDa</td>
<td>CL</td>
<td>Nuclear</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>JZB39 mAb</td>
<td>120 and 90 kDa</td>
<td>Theca and small and large luteal cells</td>
<td>Nuclear</td>
<td>Szuiki et al., 1994</td>
</tr>
<tr>
<td></td>
<td>JZB39 mAb</td>
<td>60–80% of luteinizing GC</td>
<td>Germinal epithelium, CL, cancer</td>
<td>Nuclear</td>
<td>Greenberg et al., 1990</td>
</tr>
<tr>
<td></td>
<td>JZB39 mAb</td>
<td>60–80% of luteinizing GC</td>
<td>Germinal epithelium, theca, CL, GC of preovulatory follicles</td>
<td>Nuclear</td>
<td>Zeimet et al., 1994</td>
</tr>
<tr>
<td></td>
<td>JZB39 mAb</td>
<td>60–80% of luteinizing GC</td>
<td>Germinal epithelium, theca, CL, theca, CL, GC of preovulatory follicles</td>
<td>Nuclear</td>
<td>Revelli et al., 1996</td>
</tr>
<tr>
<td></td>
<td>JZB39 mAb</td>
<td>60–80% of luteinizing GC</td>
<td>Germinal epithelium, theca, CL, theca, CL, GC of preovulatory follicles</td>
<td>Nuclear</td>
<td>Ottander et al., 2000</td>
</tr>
<tr>
<td>Baboon</td>
<td>JZB39 mAb</td>
<td>60 kDa</td>
<td>Germinal epithelium, theca, CL, GC of preovulatory follicles</td>
<td>Nuclear</td>
<td>Hild-Petito et al., 1988</td>
</tr>
<tr>
<td>Rat</td>
<td>mAb C262</td>
<td>115 and 85 kDa</td>
<td>Luteinizing GC in culture</td>
<td>Nuclear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mAb C262</td>
<td>97 and 66 kDa</td>
<td>Luteinizing GC in culture</td>
<td>Nuclear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mAb 928</td>
<td>97 and 66 kDa</td>
<td>Luteinizing GC in culture</td>
<td>Nuclear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mAb H928</td>
<td>97 and 66 kDa</td>
<td>Not present in CL of pregnancy</td>
<td>Nuclear?</td>
<td>Park-Sarge et al., 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transient expression in GC of luteinizing ovary</td>
<td></td>
<td>Park and Mayo, 1991</td>
</tr>
<tr>
<td>Pregnant rat</td>
<td>mAb LET81</td>
<td>115 and 85 kDa</td>
<td>Not present in CL of pregnancy</td>
<td>Nuclear?</td>
<td>Telleria et al., 1999</td>
</tr>
<tr>
<td>Pig</td>
<td>Novocastra mAb</td>
<td>120 and 80 kDa</td>
<td>GC and theca but not CL</td>
<td>Nuclear</td>
<td>Slomczynska et al., 2000</td>
</tr>
<tr>
<td>Bovine</td>
<td>JZB39 mAb</td>
<td>60 and 55 kDa</td>
<td>Large and small luteal cells</td>
<td>Nuclear</td>
<td>Rueda et al., 2000</td>
</tr>
<tr>
<td></td>
<td>mAb C262</td>
<td>60 and 55 kDa</td>
<td>Large and small luteal cells</td>
<td>Nuclear</td>
<td>Bramley et al., 2002</td>
</tr>
</tbody>
</table>

CL: corpus luteum; GC: granulosa cells; HOSE: human ovarian surface epithelium cells; mAb: monoclonal antibody; PR-A: progesterone receptor A; PR-B: progesterone receptor B; SIGC: spontaneously induced granulosa cells.

Epitopes: mAb C262: raised to C-terminal 14 amino acids of gPR; mAb 928: raised to hinge region of gPR; conformation-dependent; mAb JZB39: recognizes occupied and unoccupied human gPR.
2001), and (b) cloning of the receptor responsible for inducing oocyte maturation (xPR) has revealed it to be similar to the mammalian genomic progesterone receptor (Maller, 2001). xPR associates with p42 MAPK and activates oocyte maturation non-classically by signalling through the PI3-K pathway (Bagowsk et al., 2001).

Studies designed to identify and localize progesterone receptors in ovarian tissues have often used antibodies to the LBD of the gPR (Tables 5 and 6). However, antibodies raised to other regions of the gPR often give little or no signal in these same tissues (for review, see Press and Greene, 1988; El-Hefnawy et al., 2000; Luconi et al., 1998 – but see Luconi et al., 2002). Furthermore, a commonly used monoclonal antibody raised to the C-terminal end of the gPR (mAb C262) also detects immunoactivity in isolated rat granulosa cell membranes (Peluso and Pappalardo, 1998, 1999; Peluso et al., 2001) and in bovine luteal membranes and detergent extracts (Bramley et al., 2002), indicating that the membrane steroid receptor may possess a domain with homology to the LBD of the gPR. Indeed, different methods of detecting mPR can identify proteins of markedly different sizes even in the same tissues and species (for review, see Bramley et al., 2002). Finally, it will be of interest to establish whether gPR variants with deleted exons or novel 5′-regions ( vide infra ) are present in tissues with high amounts of membrane progesterone binding sites and, if so, to study their subcellular distribution at different stages of ovarian development and functional activity.

Conclusion

Progesterone can induce rapid and specific changes in a variety of tissues by acting via membrane-associated receptors. However, the nature of these receptors is presently unclear. Although membrane-associated, progesterone-specific receptors have been isolated and cloned from a range of tissues in a number of species, the protein isolated appears to be only one subunit of the (possibly dimeric) membrane receptor, and it has been difficult to demonstrate binding to the expressed protein. However, other studies indicate a membrane protein (or an associated protein) with homology to the ligand-binding domain of the genomic progesterone receptor. Further studies of these receptors in the ovary, and the ways in which they interact with other autocrine or paracrine factors, may lead to exciting advances in the understanding of the progesterone-dependent processes of ovulation, luteinization, follicular atresia and maternal recognition of pregnancy, and so permit the development of new methods of manipulating ovarian activity and fertility in humans and other mammalian species.

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