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

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

	Genomic effect	Non-genomic effect
Latency of action?	Slow (normally several hours)	Rapid (seconds to minutes)
Inhibitors of transcription?	Effect blocked	Effect persists
Cells lacking a nucleus?	No effect demonstrable	Effect persists
Cells lack genomic receptor?	No effect demonstrable	Effect persists
Isolated cell membranes?	No effect demonstrable	Effects demonstrable
Cell-impermeable steroid?	No effect demonstrable	Effect persists
Inhibitors of 'classic' genomic steroid receptor?	Effect blocked	Effect persists

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 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 Ca^{2+} and inositol trisphosphate (IP_3) (and protein kinase C $\beta 1$ (PKC- $\beta 1$)) 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 Ca^{2+} , IP_3 , 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- ^{125}I -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.

Fig. 1. Genomic structure and functional domains of the human progesterone receptor and its variants. DBD: DNA-binding domain; LBD: ligand-binding domain; PR: progesterone receptor. Arrows indicate transcription initiation sites.

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, phosphatidylinositol 3-kinase (PI3-K)) that are activated by growth factors and cytokines (Richer *et al.*, 1998; Boonyaratanakornkit *et al.*, 2001; Bagowski *et al.*, 2001). Furthermore, gPRs can exist in different functional states (Smith *et al.*, 2000), and can exhibit direct non-genomic 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 non-genomically 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

Species	Type of cell	Effective dose of		Effect	Reference
		Progestagen	Antiprogestagen		
Pig	Granulosa	1 pmol P l ⁻¹ 1 pmol P l ⁻¹	1 μmol RU486 l ⁻¹ 1 μmol RU486 l ⁻¹ 30 nmol RU486 l ⁻¹ 30 nmol ONA l ⁻¹	Increased [Ca ²⁺], IP ₃ Increased [Ca ²⁺], IP ₃ , PKC-β1 Upregulated gPR and inhibited E ₂ /P accumulation	Machelon <i>et al.</i> , 1996* [†] Lieberherr <i>et al.</i> , 1999* [†] Wu <i>et al.</i> , 1999
	Stable granulosa cell line (JC-410)	3–10 μmol LNG l ⁻¹ 10 μmol P l ⁻¹ 0.1 μmol RWJ l ⁻¹	1–10 μmol RU486 l ⁻¹	LNG and RU stimulated P accumulation. P, RU and LNG increased P450 _{scc} transcription. RWJ inhibited P450 _{scc} expression.	Swan <i>et al.</i> , 2002 [‡]
	Theca		30 nmol RU486 l ⁻¹ 30 nmol ONA l ⁻¹	Inhibited P secretion	Wu <i>et al.</i> , 1999
Sheep	Ovarian surface epithelium cells	3 nmol P l ⁻¹		Upregulated p53 and inhibited E ₂ -stimulated proliferation	Murdoch and Van Kirk, 2002 [†]
		30 nmol P l ⁻¹	60 nmol RU486 l ⁻¹	Increased PARP activity	Murdoch, 1998 [†] , Murdoch <i>et al.</i> , 2001 [†]
Cow	Granulosa (+ forskolin/insulin)	1 μmol MPA l ⁻¹	0.1 μmol RU486 l ⁻¹ 1 μmol ONA l ⁻¹	Inhibited upregulation of oxytocin gene	Lioutas <i>et al.</i> , 1997 [§]
	Cumulus–oocyte complex	10 μmol P l ⁻¹		Inhibited E ₂ secretion	Mingoti <i>et al.</i> , 2002
	Corpus luteum	50 nmol P l ⁻¹	50 nmol RU486 l ⁻¹ 5 μmol ONA l ⁻¹	P inhibited and RU and ONA stimulated apoptosis induced by AG	Rueda <i>et al.</i> , 2000 [†]
		5 μmol P l ⁻¹		Decreased PGF _{2α} and prostacyclin secretion	Pate, 1988

*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; P450_{scc}: 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

Type of cell	Effective dose range		Effect	Reference
	Progestagen	Antiprogestagen		
Granulosa (luteinizing IVF cells)	1 nmol–10 $\mu\text{mol P l}^{-1}$	50 $\mu\text{mol RU486 l}^{-1}$	Induced apoptosis and stimulated caspase-3	Svensson <i>et al.</i> , 2001 [§]
	10 nmol P l^{-1}	10 $\mu\text{mol ORG}$		
		1 $\mu\text{mol RU486 l}^{-1}$	P reduced apoptosis and had protective effect on survival	Makrigiannakis <i>et al.</i> , 2000 [†]
		50 $\mu\text{mol RU486 l}^{-1}$	Reduced secretion of (E_2) P and relaxin	VanderVoort <i>et al.</i> , 2000
		50 $\mu\text{mol HRP l}^{-1}$		
		5–10 nmol RU486 l^{-1}	Suppressed P secretion	DiMattina <i>et al.</i> , 1986
		50–500 $\mu\text{mol RU486 l}^{-1}$	Inhibited 17-hydroxylase. No effect on E_2 secretion	DiMattina <i>et al.</i> , 1987
Ovarian surface epithelium cells		10–100 nmol RU486 l^{-1}	No effect on basal, hCG- or PGE_2 -stimulated P	Greenberg <i>et al.</i> , 1990
	640 nmol P l^{-1}	64 nmol RU486 l^{-1}	P inhibited proliferation and differentiation	Chaffkin <i>et al.</i> , 1993 [†]
	160 nmol P l^{-1}	100 nmol ORG l^{-1}	Suppressed proliferation. Enhanced proliferation	Ivarsson <i>et al.</i> , 2001 [†]
		10 nmol RU486 l^{-1}	Inhibited hCG-stimulated (but not basal) P secretion	Ottander <i>et al.</i> , 2000
Corpus luteum				
SKOV-3 ovarian cancer	30–300 nmol P l^{-1}	3 $\mu\text{mol RU486 l}^{-1}$	Inhibited uPA secretion and cell invasiveness	McDonnell and Murdoch, 2001 ^{*‡}
gPR-positive ovarian carcinoma cell lines		30–120 nmol RU486 l^{-1}	Inhibited proliferation (blocked G_0/G_1 phase of cell cycle) and downregulated gPR	Rose and Barnea, 1996

*Demonstration of cell surface receptor action.

[†]Progestin effect reversed by gPR antagonist.

[‡]Progestin effect not reversed by gPR antagonist.

[§]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

Type of cell	Effective dose range		Effect	Reference
	Progestagen	Antiprogestagen		
Granulosa cells	1 $\mu\text{mol P l}^{-1}$		Enhanced LH- and FSH-stimulated steroidogenesis	Fanjul <i>et al.</i> , 1983
	1 $\mu\text{mol R5020 l}^{-1}$			
		300 nmol RU486 l^{-1}	Inhibited aromatase. Inhibited P secretion	Fortune and Vincent, 1983
		30 $\mu\text{mol RU486 l}^{-1}$		
	200 $\mu\text{mol P l}^{-1}$	64 $\mu\text{mol RU486 l}^{-1}$	P reduced and antagonist enhanced apoptosis and cell adhesion	Peluso and Pappalardo, 1994 [†]
		25 $\mu\text{mol RU486 l}^{-1}$	Antagonized hCG-induced apoptosis and suppressed expression of gPR	Svensson <i>et al.</i> , 2000
Large granulosa cells		10 $\mu\text{mol ORG l}^{-1}$		
		1 $\mu\text{mol ONA l}^{-1}$	Inhibition of hCG- and forskolin-induced PACAP mRNA expression	Ko <i>et al.</i> , 1999
	640 nmol P l^{-1}	64 $\mu\text{mol RU486 l}^{-1}$	P inhibited apoptosis of large GC	Luciano <i>et al.</i> , 1994 [†]
	64 nmol P l^{-1}		Stimulated bFGF secretion and inhibited apoptosis in large luteal cells	Peluso and Pappalardo, 1999*
Small granulosa cells	160 nmol P l^{-1}	64 $\mu\text{mol RU486 l}^{-1}$	P inhibited insulin-stimulated mitosis	Luciano and Peluso, 1995 [†]
Granulosa/SIGC	640 nmol P l^{-1}	640 nmol RU486 l^{-1}	Inhibited apoptosis and MAPKK	Peluso <i>et al.</i> , 2001* [‡]
		640 nmol ONA l^{-1}		
Luteinizing granulosa cells		10 $\mu\text{mol ONA l}^{-1}$	Inhibited hCG and forskolin on PAC-1 receptor expression	Ko and Park-Sarge, 2000
	150 nmol P l^{-1}	2 $\mu\text{mol ONA l}^{-1}$	Induced GRE expression; RU486 and ONA antagonized P effect, but did not block LH-induction of gPR	Natraj and Richards, 1993 [†]
		2 $\mu\text{mol RU486 l}^{-1}$		
CL cells	10 $\mu\text{mol LNG l}^{-1}$		Inhibited LH and cAMP stimulated P secretion	Telleria and Deis, 1994
CL of pregnancy	0.1 $\mu\text{mol P l}^{-1}$		Suppressed IL-6 expression	Telleria <i>et al.</i> , 1998
	1–100 $\mu\text{mol R5020 l}^{-1}$		Stimulated P secretion	Telleria <i>et al.</i> , 1999
CL and SV40 sensitive cell line	10 nmol P l^{-1}	1 $\mu\text{mol RU486 l}^{-1}$	Inhibited 20 α -HSD expression	Sugino <i>et al.</i> , 1997 ^{†§}
CL-derived phagocytes	300 nmol P l^{-1}	100 nmol RU486 l^{-1}	Inhibited superoxide production	Sugino <i>et al.</i> , 1996 [†]

*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; 20 α -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.

Fig. 2. Genomic and non-genomic activation pathways of progesterone. DAG: diacylglycerol; GABA: gamma aminobutyric acid; MAPK: mitogen-activated protein kinase; MEK: mitogen-activated (extracellular) protein kinase kinase; P: progesterone; PI3-K: phosphatidylinositol 3-kinase; PKA: protein kinase A; PKC: protein kinase C; PLC: phospholipase C; PTK: protein tyrosine kinase.

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 Ca²⁺ mobilization (influx or mobilization from intracellular stores), opening of Na⁺ and Cl⁻ channels, and the activation of phospholipase C (leading to increased intracellular IP₃ 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,

Table 5. Studies demonstrating expression of the gene for genomic progesterone receptor in ovarian tissues

Species	Primers (nucleotides)	Method	Type of cell	Reference
Human	2113–2343	RT–PCR (PR-B)	CL	Ottander <i>et al.</i> , 2000
	144–367	RT–PCR (PR-A/B)		
		Southern blots/ISH		
	1341–1360	RT–PCR/Southern blot	CL	Misao <i>et al.</i> , 1998a
Monkey	PR-A and PR-B	Quantitative RT–PCR	CL	Misao <i>et al.</i> , 1998b
	1817–36 and 2330–49	Southern blot	HOSE cells	Lau <i>et al.</i> , 1999
	LBD	RT–PCR, sequencing, restriction enzyme cleavage	Germinal epithelium, CL, luteinizing GC	Chandrasekher <i>et al.</i> , 1994
	LBD	RPase	CL, luteinized GC	Duffy <i>et al.</i> , 1996
Baboon	Full length hPR	Northern blot	CL	Duffy and Stouffer, 1995
Bovine		RPase/northern blot	CL	Hild-Petito and Fazleabas, 1997
		RT–PCR, sequencing	GC	Lioutas <i>et al.</i> , 1997
	Hinge/LBD	Northern blot	CL	Rueda <i>et al.</i> , 2000
			Periovulatory follicle	Cassar <i>et al.</i> , 2002
Rat	LBD	RT–PCR, ISH	GC of preovulatory follicles	Park-Sarge <i>et al.</i> , 1995
	LBD	RT–PCR	Transient expression in GC of luteinizing primed ovary	Park and Mayo, 1991

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; RPase: ribonuclease protection assay.

Table 6. Studies demonstrating genomic progesterone receptor protein expression in ovarian tissues

Species	Antibody	Protein detected	Type of cell	Subcellular localization	Reference
Human	Range of mAbs	116 and 97 kDa	Ovarian tumours, CL, stroma, HOSE GC of dominant follicles	Nuclear	Press and Green, 1988 Iwai <i>et al.</i> , 1990
	mAb C262	120 and 90 kDa	CL	Nuclear?	Misao <i>et al.</i> , 1998a
	mAb C262	116 and 90 kDa	CL		Misao <i>et al.</i> , 1998b
	Neomarkers hPRa3	PR-B and PR-A	Ovarian cancers	Nuclear	Akahira <i>et al.</i> , 2000
	Neomarkers hPRa7	PR-A			
	Neomarkers hPRa2	PR-B			
	Novocastra mAb	120 and 90 kDa	Theca and small and large luteal cells	Nuclear	Suzuki <i>et al.</i> , 1994
	JZB39 mAb		60–80% of luteinizing GC	Nuclear	Greenberg <i>et al.</i> , 1990
	Abbott K68		Germinal epithelium, CL, cancer	Nuclear	Zeimet <i>et al.</i> , 1994
	Abbott		CL (theca; GC of some follicles)		Revelli <i>et al.</i> , 1996
Monkey	Abbott		CL	Nuclear	Ottander <i>et al.</i> , 2000
	JZB39 mAb		Germinal epithelium, stroma, theca, CL, GC of preovulatory follicles	Nuclear	Hild-Petito <i>et al.</i> , 1988
Baboon	JZB39 mAb		Luteinized GC	Nuclear	Duffy <i>et al.</i> , 1996
	JZB39 mAb		CL	Nuclear	Hild-Petito and Fazleabas, 1997
Rat	mAb C262	60 kDa	GC/SIGC	Cell membrane	Peluso and Pappalardo, 1998
	mAb C262		Small GC	Cell membrane	Peluso <i>et al.</i> , 2001
	mAb 928	115 and 85 kDa	Luteinizing GC in culture	Nuclear	Natraj and Richards, 1993
	mAb 928	97 and 66 kDa			
	mAb H928		Not present in CL of pregnancy Transient expression in GC of luteinizing ovary		Park-Sarge <i>et al.</i> , 1995 Park and Mayo, 1991
Pregnant rat	mAb LET81		GC and theca but not CL	Nuclear?	Telleria <i>et al.</i> , 1999
Pig	Novocastra mAb	120 and 80 kDa	CL, theca, GC of preovulatory follicles	Nuclear	Slomczynska <i>et al.</i> , 2000
Bovine	JZB39 mAb		Large and small luteal cells	Nuclear	Rueda <i>et al.</i> , 2000
	mAb C262	60 and 55 kDa	CL	Cell membrane	Bramley <i>et al.</i> , 2002

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 (Bagowski *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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Key references are identified by asterisks.

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