Review

Molecular mechanisms of steroid receptor-mediated actions by synthetic progestins used in HRT and contraception

Donita Africandera, Nicolette Verhoogb, Janet P. Hapgoodb,∗

a Department of Biochemistry, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa
b Department of Molecular and Cell Biology, University of Cape Town, Private Bag X3, Rondebosch, 7701, South Africa

1. Introduction

Progestins are classified as compounds that transform proliferative endometrium to secretory endometrium in estrogen-primed uteri[1]. Progesterone (Prog), the natural progestin in humans, is critical for female reproductive function. When Prog levels decline,

2. Classification and structure of progestins: old vs. new

3. Steroid receptor-mediated mechanisms of action

3.2. Relevant pharmacological parameters and pharmacokinetic considerations

3.3. Effects of old vs. new synthetic progestins on target genes via the:

3.3.1. Progesterone receptor (PR)

3.3.2. Glucocorticoid receptor (GR)

3.3.3. Androgen receptor (AR)

3.3.4. Mineralocorticoid receptor (MR)

3.3.5. Estrogen receptor (ER)

4. Conclusions

© 2011 Elsevier Inc. All rights reserved.

* Corresponding author. Tel.: +27 21 650 5977; fax: +27 21 6897573.
E-mail address: Janet.Hapgood@uct.ac.za (J.P. Hapgood).

0039-128X/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2011.03.001
menstruation and endometrial repair occur [2], while increased plasma levels of Prog result in a lack of ovulation during pregnancy [3]. This inhibitory effect of Prog on ovulation, as well as the local changes in the cervical mucus induced by antiestrogens, to inhibit sperm penetration, are the basis for the development of synthetic progestins as contraceptives. Due to the rapid metabolism and resulting short biological half-life of Prog [4], its use as a contraceptive is limited. Progestins are also used in hormone replacement therapy (HRT), and in a number of other therapeutic applications such as treatment of gynaecological disorders and in cancer therapy. HRT includes administration of either estrogen alone, or estrogen combined with a progestin [5]. The rationale for progestin and estrogen usage for contraception is very different to that for HRT usage. For contraception, the primary goal is to prevent pregnancy, and thus potent progestins are used to inhibit ovulation and sperm penetration. For HRT however, progestins are used in menopausal women with an intact uterus, to counteract the proliferative effects of estrogen on the uterine epithelium, thereby preventing estrogen-induced endometrial hyperplasia [6,7]. Thus contraception aims to inhibit physiological mechanisms, while HRT aims to maintain or restore a physiological status [8]. For contraception, progestins are usually combined with estrogen for better cycle control, or used alone as progestin-only contraceptives, while for HRT, estrogen is used to prevent the side-effects associated with lack of estrogen [9].

Progestins, a class of synthetic steroids structurally distinct, but functionally similar to Prog, with longer biological half-lives, were first synthesized more than 50 years ago [4]. These first generation progestins include medroxyprogesterone acetate (MPA) and norethisterone enanthate (NET-EN), which are the most widely used injectable female contraceptives, with at least 20 million current users of MPA worldwide [10]. Today, a wide variety of synthetic progestins is available, that in addition to their common progestogenic effects, exhibit a range of biological activities that differ not only from each other, but also from that of Prog [11].

However, a number of side-effects have been reported with the clinical use of progestins [12,13]. The importance of investigating their molecular mechanisms of action is highlighted by clinical evidence showing that MPA and NET, in combination with estrogen, increase the risk of breast cancer and that MPA increases the risk of cardiovascular complications in long term HRT users [14,15]. Besides the above complications, several other side-effects have been reported with usage of progestins in contraception or HRT, such as a modest but significant increase in shedding of HIV-1 DNA in humans [16], effects on bone density [17–19], blood pressure [20,21] immune function [22–25], neurological effects [26,27] and more minor effects such as mood swings, weight gain, hot flushes and loss of libido [13–15,25,28–31]. While some [11,32,33], but not other [12] progestins have been reported to exhibit differential side-effects, there is evidence that the choice of specific progestin or dosage thereof could determine risk outcome [34–36]. Different risk outcomes would most likely be due to differences in dosage, metabolism, pharmacokinetics, bio-availability, binding affinities and specificities for serum proteins as well as different affinities, specificities and biological activities via different steroid receptors or receptor isoforms [1,11,33,37–39].

At the cellular level, progestins mediate their effects via alterations in transcription of specific genes in target cells predominantly via binding to and regulating the activity of steroid receptors, which are ligand-activated transcription factors. Although the progestational effects of all progestins are generally considered to be mediated by binding to the progesterone receptor (PR) in female reproductive tissue [40], many of their side-effects are most likely due to binding to other members of the steroid receptor family in non-reproductive tissues. For example, the increased blood pressure, weight gain and risk of cardiovascular disease are most likely due to lack of mineralocorticoid receptor (MR) antagonism in the colon and kidneys, while negative effects on bone density and immune function are most likely mediated by glucocorticoid receptor (GR) agonism [41–45]. Thus the physiological effects of a particular progestin may be influenced by cell-specific expression of different levels of steroid receptors and their isoforms [33]; reviewed in [37]. Another factor complicating the prediction of the physiological effects of progestins is the presence of plasma membrane steroid receptors that signal by rapid non-genomic mechanisms and crosstalk between various signaling pathways [46–49].

This review will focus on the mechanisms of action of progestins via different steroid receptors. It aims to highlight the differences between old vs. new generation synthetic progestins, as compared to Prog, in terms of binding to different steroid receptors, as well as their effects on target genes via these steroid receptors, with a view to improved understanding of the physiological outcomes of these progestins in vivo, and identifying new areas of research.

### 2. Classification and structure of progestins: old vs. new

Progestins are classified according to successive generations, and they differ in terms of their structures and receptor selectivities (Table 1). It appears generally accepted that a synthetic progestin should act like Prog, and be a potent PR agonist, and exhibit no interaction with the androgen receptor (AR), glucocorticoid receptor (GR) and estrogen receptor (ER) [9]. However, these considerations do not appear to have been taken into account when the early generation progestins were developed. Examples of first generation synthetic progestins include medroxyprogesterone acetate (MPA), a 17-α-progestin derivative (21-carbon series steroid) containing the pregnane nucleus, and norethisterone (NET) and its derivatives norethisterone acetate (NET-A) and norethisterone enanthate (NET-EN), 19-nortestosterone derivatives containing the androstanone nucleus. Due to the aforementioned structures, MPA is often referred to as a true progestin, while NET-EN, which retains its androgenic activity, is referred to as an androgenic progestin [50]. An example of a second generation progestin is levonorgestrel (LNG), while third generation progestins, developed to decrease androgenic activity [51], include derivatives of LNG such as gestodene (GES). LNG and GES are both 19-nortestosterone derivatives, similarly to NET-EN. Unlike the older progestins, most of the fourth generation progestins have been designed to be closer in activity to natural Prog. The term activity, in this context, usually refers to measured biological activity in animal models, which does not necessarily correlate with an established role for a particular steroid receptor. Prog has been reported to lack androgenic or estrogenic activity [11,52,53], while possessing anti-estrogenic and anti-mineralocorticoid properties, as well as weak glucocorticoid-like properties [54–56]. However, some of the biological activities reported in the literature are not consistent (Table 2). For example, some report that Prog has weak androgenic properties, and no glucocorticoid activity.

The newer progestins include drospirenone (DRSP), dienogest (DNG) and trimegestone (TMG). DRG is derived from spironolactone, the well-known mineralocorticoid receptor (MR) antagonist [57]. It has the 19-carbon structure of the parent compound, androstane, with a carbolacone group attached to C-17 [53,58]. DNG is a 19-nortestosterone derivative, but differs from other nortestosterone derivatives by its cyanoamethyl instead of an ethynyl group at C-17 [11]. TMG is a 19-norpregnane progestin [59], and is the most potent progestin in terms of the endometrial transformation test in the rabbit [60]. Nestorone (NES) and nomogesterone acetate (NOMAc), both 19-norpregnanes like TMG, were designed to have high selectivity for binding to the PR, with little or no
Table 1
Relative binding affinities (RBAs) of progesterone and synthetic progestins to steroid receptors.

<table>
<thead>
<tr>
<th></th>
<th>PR</th>
<th>MPA</th>
<th>NET-A</th>
<th>LNG</th>
<th>GES</th>
<th>DNG</th>
<th>DRSP</th>
<th>TMG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PR</strong></td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>298&lt;sup&gt;b&lt;/sup&gt;; 230&lt;sup&gt;b&lt;/sup&gt;</td>
<td>134&lt;sup&gt;c&lt;/sup&gt;; 150&lt;sup&gt;b&lt;/sup&gt;</td>
<td>323&lt;sup&gt;c&lt;/sup&gt;; 300&lt;sup&gt;b&lt;/sup&gt;</td>
<td>864&lt;sup&gt;c&lt;/sup&gt;; 180&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19&lt;sup&gt;b&lt;/sup&gt;; 70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>588&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>GR</strong></td>
<td>11&lt;sup&gt;c&lt;/sup&gt;; 6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>58&lt;sup&gt;b&lt;/sup&gt;; 29&lt;sup&gt;b&lt;/sup&gt;; 79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.4&lt;sup&gt;c&lt;/sup&gt;; 0&lt;sup&gt;b&lt;/sup&gt;; 0.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.5&lt;sup&gt;d&lt;/sup&gt;; 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38&lt;sup&gt;c&lt;/sup&gt;; 27&lt;sup&gt;b&lt;/sup&gt;; 56&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3&lt;sup&gt;b&lt;/sup&gt;; 1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>AR</strong></td>
<td>3&lt;sup&gt;b&lt;/sup&gt;; 2&lt;sup&gt;d&lt;/sup&gt;; 80&lt;sup&gt;e&lt;/sup&gt;</td>
<td>35&lt;sup&gt;b&lt;/sup&gt;; 5&lt;sup&gt;e&lt;/sup&gt;; 151&lt;sup&gt;d&lt;/sup&gt;; 75&lt;sup&gt;d&lt;/sup&gt;</td>
<td>55&lt;sup&gt;b&lt;/sup&gt;; 15&lt;sup&gt;b&lt;/sup&gt;; 134&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58&lt;sup&gt;b&lt;/sup&gt;; 45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71&lt;sup&gt;b&lt;/sup&gt;; 85&lt;sup&gt;b&lt;/sup&gt;; 33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5&lt;sup&gt;b&lt;/sup&gt;; 65&lt;sup&gt;b&lt;/sup&gt;; 0.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>MR</strong></td>
<td>1000&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;c&lt;/sup&gt;; 100&lt;sup&gt;b&lt;/sup&gt;; 134&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;d&lt;/sup&gt;; 0&lt;sup&gt;c&lt;/sup&gt;; 0.005&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17&lt;sup&gt;c&lt;/sup&gt;; 73&lt;sup&gt;d&lt;/sup&gt;</td>
<td>97&lt;sup&gt;c&lt;/sup&gt;; 290&lt;sup&gt;d&lt;/sup&gt;; 11&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>500&lt;sup&gt;c&lt;/sup&gt;; 230&lt;sup&gt;d&lt;/sup&gt;; 100&lt;sup&gt;d&lt;/sup&gt;</td>
<td>42&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>ER</strong></td>
<td>0.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.02&lt;sup&gt;c&lt;/sup&gt;; 0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.15&lt;sup&gt;c&lt;/sup&gt;; 0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.02&lt;sup&gt;c&lt;/sup&gt;; 0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>0.5&lt;sup&gt;c&lt;/sup&gt;; 0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

RBAs are expressed as a percentage of total specific binding, with that of the natural ligand<sup>a,b,c</sup>, or synthetic agonist<sup>d</sup>,<sup>e</sup> of each receptor set at 100%. ND not determined. Prog: progesterone; 4-pregnene-3,20-dione. MPA: medroxyprogesterone acetate (6α-methyl-17α-acetoxy pregn-4-en-3,20-dione), also referred to as Depo-Provera<sup>®</sup>, depot medroxyprogesterone acetate (DMPA) when used as an oily suspension for injection. NET-A: (R = OCOCH<sub>3</sub>) norethisterone acetate (17α-ethynyl-19-nortestosterone 17β-acetate); also used as NET (R = OH) norethisterone (19-nor-17α-ethynylestosterone) or NET-EN (R = OCO(CH<sub>2</sub>)<sub>5</sub>CH<sub>3</sub>) norethisterone enanthate (7α-ethynyl-17β-heptanoyloxy-4-estren-3-one), the latter also referred to as either norethindronenanthate, norethisterone enanthate or Nur-Isterate, for injectable contraception. LNG: levonorgestrel (3β-ethyl-17α-ethynyl-17β-hydroxy-4-en-3-one). GES: gestodene (17α-ethinyl-17β-hydroxy-18-methylene-4,15-dien-3-one). DNG: dienogest (17α-cyanomethyl-17β-hydroxyestra-4,9-dien-3-one). DRSP: drospirenone (6β, 7β, 15β, 16β-dimethylene-3-oxo-17α-pregn-4-en-21,17-carbolactone). TMG: trimigestone 17β-[(S)-2-hydroxypropanoyl]-17-methyl-estra-4,9-dien-3-one.

For each receptor, the following abbreviations are used:
- **PR**: progesterone receptor
- **GR**: glucocorticoid receptor
- **AR**: androgen receptor
- **MR**: mineralocorticoid receptor
- **ER**: estrogen receptor

<sup>a</sup> Determined by recombinant human steroid receptor binding in vitro [60].
<sup>b</sup> Means of RBA determination not defined [11].
<sup>c</sup> RBAs were determined by overexpressing the hGR in COS-1 cell line [171].
<sup>d</sup> RBAs were determined by overexpressing the hAR and hMR in COS-1 cells [our unpublished data].
<sup>e</sup> RBA was determined by overexpressing the hAR in COS-1 cells [189].
interaction with other steroid receptors [9]. A number of clinical studies have indicated that NOMAc, in combination with 17β-estradiol (E2), is an effective contraceptive agent, with a positive safety and tolerability profile [reviewed in [56]]. Current progestins thus include a wide range of progestogenic molecules with variable degrees of estrogenic, androgenic, anti-androgenic, glucocorticoid-like, and anti-mineralocorticoid activity. The choice of progestin can thus be determined based on the particular requirements for contraception, or HRT, as well as particular risk factors of the patient.

Although the structure-function relationships that determine receptor selectivities and affinities of different progestins are not always obvious, clearly the chemical structure of the progestin and the precise amino acids in the ligand binding domain of a steroid receptor influence ligand binding affinity, specificity and transcriptional activity of a particular progestin-bound receptor. A detailed review of this topic is beyond the scope of this review. In general, steroid selectivity appears to be influenced by the complementarity of shape and hydrogen bonding between ligands and the receptor ligand-binding pocket [61]. Regions encompassing helices 6 and 7 of steroid receptors have been shown to be key determinants of ligand binding and transactivation potential of steroid receptors [62]. Although the steroid receptors share considerable homology in amino acid sequence and three-dimensional structure, many subtle differences exist in the secondary structure and topology of the ligand-binding pockets of these receptors [62,63], which could explain the differential selectivity of progestins for different steroid receptors.

### 3. Steroid receptor-mediated mechanisms of action

#### 3.1. General mechanisms of action of steroid receptors

The effects of steroid hormones are mediated through binding to intracellular steroid receptors, a subfamily of the nuclear receptor superfamily. The steroid receptor family comprises the PR, GR, AR, MR, and ER, hormone-activated transcription factors that share a high level of similarity with regards to their structures, as well as their mechanisms of action. Steroid receptors consist of distinct domains namely: the highly variable transcriptional activation function-1 domain (AF-1), the highly conserved zinc finger DNA-binding domain (DBD), and the moderately conserved C-terminal ligand-binding domain (LBD) (Fig. 1). The amino terminal (A/B) domain is much less conserved between the steroid receptors and differs in both length (Fig. 1) and amino acid sequence [64]. The high variability of this domain is believed to be instrumental to the specificity of a steroid receptor’s response as it influences cofactor-LBD interactions [64]. The ligand independent AF-1 domain situated near the N-terminus has been reported to be responsible for protein–protein interaction with general transcription factors as well as cofactors [65]. It is also required for optimal transcriptional activity (reviewed by [65]). The DBD, which is highly homologous between the steroid receptors, plays an important role in receptor dimerization, DNA-binding specificity, and interaction with cofactors [66]. Receptor dimerization is also mediated in part through the LBD [67] and the nuclear localization signal is embedded in both the DBD and LBD [68]. The LBD is also involved in protein–protein interactions with chaperone proteins [69]. Subtle differences however exist between dimerization and cofactor binding of the steroid receptors, in particular between the ER and the other steroid receptors and is attributed to the secondary structure of the receptors [63,70,71]. The LBDs of the PR, GR, AR and MR share about 50–57% sequence homology, while the ER-LBD has only 28% homology with the PR-LBD [72–74]. Within the LBD, a cofactor docking site (activation function-2 or AF-2 domain) is present, which is highly conserved and important for the induction of transcriptional activity of the receptor [67]. AF-2 transcriptional activity is dependent on ligand, unlike AF-1 transcriptional activity, which is able to act independent of ligand [67]. The AF-2 domain also contains the specific LxxLL motif that interacts with certain cofactors [75], and which is not present in the highly variable AF-1 domain [65].

In the absence of ligand, the AR, GR and MR are present predominantly in the cytoplasm whereas the ER and PR are present predominately in the nucleus [66] in an inactive state in a protein complex with heat shock proteins (hsp)-90 and hsp70, immunophillins, and other factors [76]. Hsp90 acts as a molecular chaperone, and prevents unliganded steroid receptor dimerization [77]. The ligand binding domain of the steroid receptor occurs, followed by rapid nuclear translocation in some cases [66,78]. Steroid receptors are hyperphosphorylated upon ligand binding, which is generally associated with a transcriptionally active steroid receptor. In the nucleus, the ligand-bound steroid receptor binds as a dimer to specific palindromic DNA sequences known as steroid responsive elements (SREs), followed by chromatin remodelling, recruitment of cofactors and components of the transcriptional machinery, thereby positively regulating transcription [66,69,79,80], a process known as transactivation (Fig. 2). For example, the PR binds to SREs called progesterone response elements (PREs). Due to the high degree of structural and functional conservation within the DBD of steroid receptors, most steroid receptors can bind the same DNA response...
element (reviewed in [81]). The classical PRE thus also serves as a response element for the GR, AR and MR (reviewed in [82]), and is then termed glucocorticoid response element (GRE), androgen response element (ARE) and mineralocorticoid response element (MRE), respectively.

Steroid receptors can also negatively regulate transcription by either directly or indirectly interfering with other DNA-bound transcription factors such as nuclear factor-kappa B (NFkB) or activator protein-1 (AP-1) and CCAAT-enhancer-binding protein (C/EBP) [49,79,83–85]. This process is usually referred to as transrepression (Fig. 2). By far the best-studied in terms of transrepression is the GR (reviewed in [86]). Transrepression can take place without direct DNA binding by the receptor but rather via binding of a GR monomer to NFkB or AP-1, a process usually referred to as tethering [87–89]. As for transactivation, transrepression also involves protein–protein interactions in multiprotein complexes on the DNA and modulation of chromatin structure, although these mechanisms are less understood than for transactivation [49,79,83–85]. Many genes involved in the inflammatory response, such as cytokine and chemokine genes, have been reported to be repressed in this way by the GR [86]. Much less is known about the mechanisms by which the AR, PR and MR repress genes, but given the similar mechanisms of action established for different steroid receptors thus far for transrepression, it is likely that they repress genes by mechanisms similar to those of the well-studied GR. Indeed, NFkB can be antagonised by the PR [90] and the AR [91,92], resulting in repression of genes. The MR has been shown to repress transcription via NFkB [93] but not AP-1 in response to aldosterone (Ald), suggesting receptor-specific mechanisms of repression may occur. Besides the above-mentioned mechanisms, several other less-studied mechanisms of steroid-receptor-mediated regulation of transcription have been identified, including repression by direct DNA-binding and activation by tethering [94].

Whether activation or repression of transcription occurs depends also on the nature of the cofactors recruited by the liganded steroid receptor, which in turn depends on whether the ligand is an agonist or an antagonist. In general, an agonist bound to a receptor induces a conformational change that facilitates co-activator binding [95]. Co-activators are responsible for transcriptional activation due to their intrinsic histone acetylase activity, resulting in the opening of the chromatin structure and recruitment of basal transcription machinery [95]. In contrast, the conformational change induced by an antagonist is generally accepted to result in the recruitment of co-repressors, which results in a decrease or no transcriptional activity [96]. Transcriptional repression mediated by co-repressors is due to their histone deacetylase activity, in addition to them also recruiting other histone deacetylase complexes, thereby inhibiting chromatin remodelling [97]. Tissue-selective expression profiles of these cofactors result in differential recruitment of co-activators vs. co-repressors, leading to tissue-specific steroid responses [95].

An additional level of complexity is introduced by the findings that steroid receptors can modulate cell signaling by cross talk with other receptors and cytoplasmic signaling pathways by so-called “non-genomic” signaling, in the absence of direct effects on transcription by the steroid receptor [98–101]. Non-genomic signaling via steroid receptors has been suggested to play a role in induction of oocyte maturation, altering reproductive signaling in the brain and rapid activation of breast cancer cell signaling for the PR [101], neuroprotection for the GR and ER [102,103], maintenance of spermatogenesis for the AR [104], and signaling in the brain, as well as endothelial dysfunction and inflammation for the MR [100]. For example, progestins have been shown to activate various signaling pathways such as the MAPK and PI3-K/Akt signaling cascades [105]. This non-genomic signaling by progestins is primarily thought to be mediated by the classic PR [106]. However, some studies have reported the possible involvement of the recently identified cell surface receptor membrane PR (mPR) [107]. Although very few studies have investigated the mechanisms of synthetic progestin signaling via the mPR, one study suggests that synthetic progestins like MPA and NET, unlike Prog, do not signal via the mPR [107–109], providing another mechanism for different biological responses of progestins.

Thus many downstream signaling partners modulate the activity of steroid receptors in multistep processes, with the first step being the binding of the progestin to the receptor, as determined by its affinity for the receptor. This affinity thus contributes towards the potency of a ligand [110,111]. We will briefly provide an explanation on some pharmacological definitions [112] that are referred to in this review.
3.2. Relevant pharmacological parameters and pharmacokinetic considerations

The effect of a given ligand depends on the fraction of receptors that are occupied by the specific ligand. Fractional occupancy describes the relative receptor occupancy for any ligand at equilibrium, as a function of the concentration of ligand and the affinity of the particular ligand for the receptor. The relative binding affinity (RBA), also referred to as the IC$_{50}$ or EC$_{50}$, is a measure of the concentration of ligand that competes for half the total specific binding. It is used to estimate the binding affinity of a ligand for a receptor. The EC$_{50}$ values obtained from competitive binding curves however, are not equivalent to $K_d$ (the equilibrium dissociation constant) or $K_i$ (the equilibrium dissociation constant of a ligand determined by inhibition studies). The EC$_{50}$ is a relative term, which is influenced by different experimental conditions, such as receptor concentration and concentration of radiolabelled ligand used in the determination, while the $K_d$ or $K_i$ is a constant value for a particular receptor, which is not influenced by these variables [113]. However the relationship between affinity and biological activity or potency is not straightforward or predictable, and appears be cell- and promoter-specific, as well as ligand-dependent. The nature and extent of a response depends in particular on the milieu of coactivators and corepressors and other interacting partners of steroid receptors present in the cells [111].

To characterize the biological effects mediated by receptors, two parameters need to be determined by dose response analysis, namely efficacy and potency (Fig. 3). The efficacy of a ligand refers to the maximal effect it can elicit in a given cell under defined experimental conditions. Efficacy is used to characterize ligands as either full agonists or partial agonists. An agonist is a ligand that produces a response of similar efficacy to that of the endogenous ligand, while a partial agonist is a ligand eliciting a response less than the maximal response of an agonist (Fig. 3). In contrast, an antagonist is a compound that binds to a receptor but that does not elicit a response itself, but inhibits agonist-mediated responses (Fig. 3).
The potency of a ligand is commonly quantified as the EC50, which is the concentration that induces half the maximal response. Note that all partial agonists could potentially exhibit some antagonist activity towards a particular receptor, depending on the relative concentrations of the agonist and partial agonist. This is relevant to progestin action since several progestins are partial agonists for receptors other than the PR.

Efficacies and potencies for gene expression by progestins are highly relevant physiologically since they are a measure of the biological response for particular concentrations of progestins found in the serum, or intracellularly, in patients using them for reproductive intervention. Dose response analysis also allows prediction of how the responses changes with changing concentrations of these progestins [114]. Note that potency is cell-specific and promoter-specific [111] and will also change with changing receptor concentrations [115]. The free concentrations of progestins in serum would depend on the concentrations of serum binding proteins [11,116], the latter's affinity for progestins as well as the concentrations and affinities of endogenous competing steroids. The extent of intracellular receptor occupancy would also naturally depend on the concentrations and affinities of endogenous competing steroids for binding to the receptor, as well as the nature and extent of steroid metabolism in the cell. Given the critical dependency of response on ligand concentration, it is important to determine progestin concentrations in the serum of women undergoing treatment. When used as injectable contraceptives, levels of drug-related material have been reported to reach 25 ng/ml (65 nM) a few days post injection for MPA [117], while for NET-EN serum concentrations in the range of 1.5–59 nM [118] have been reported. Serum levels of progestins also vary depending on time after administration [1]. For example, seven days after the intramuscular injection of 150 mg Depo-Provera, serum levels of MPA range from 1750 to 9000 pg/ml and by 75 days these levels decrease to about 680–2600 pg/ml [119]. Such changes in concentration would undoubtedly affect receptor occupancy and hence physiological effects.

For use in HRT, typical serum concentrations for MPA are not easily found in the literature, while for women receiving a typical HRT regime of NET, peak serum concentrations are in the range 1.4 and 6.8 ng/ml (3.64–17.7 nM). Surprisingly, few studies have addressed the differences in metabolism and pharmacokinetics of different progestins, which may depend on the therapeutic dosage and the route of administration, as well as intra-subject variability

1. For example, administering a 10 mg tablet of MPA showed mean peak levels ranging from 3 to 5 ng/ml (about 8–13 nM) within 1–4 h post-treatment, while oral administration of 1 mg NET or 0.25 mg LNG, both showed a peak level of 6 ng/ml (about 15 nM) within 1–3 h [120–122]. Interestingly, for lower, or equivalent oral doses of DNG and DRSP, much higher serum levels were observed [123,124].

The route of progestin delivery would also affect the side-effect profile of progestins via steroid receptors. Besides oral and injectable administration of progestins, several other routes of delivery of progestins have been or are being developed for both contraception and HRT [125–128]. These include vaginal gels or rings, intrauterine devices, and transdermal patches, skin gels and sprays. It is thought that delivering the lowest possible dose of progestin or Prog directly to the uterus would avoid side-effects on other targets. Non-oral routes also avoid the first-pass effect of steroidal hormones on the liver, which can result in metabolites which may have different activities and side-effects compared to the parent compound [1]. Some progestins, like NES, are orally inactive but highly potent when administered parenterally [129]. NES has been shown to exert a high contraceptive effect when administered via routes such as vaginal rings, implants and transdermal systems. As NES has the highest anti-ovulatory action among existing progestins, a very low dose can be used and delivered from non-oral delivery systems.

Another factor that can influence the biological response to synthetic progestins is the nature, dose and formulation of the estrogen component. Conjugated oestrogens can easily enter the hepatocytes but are hormonally active only after hydroxylation into the parent steroids [8]. For contraception, progestins are in most cases used in combination with ethinyl estradiol (EE), while for HRT it is mostly natural estradiol [9,128,130]. It is generally accepted that EE should not be used for HRT since it exerts strong effects on hepatic metabolism and inhibits metabolising enzymes [8,128]. The type and concentration of estrogen also influences progestational effects of progestins, since estrogens regulate expression of the PR [128], and hence the sensitivity of the cell to progestins.

It is also possible that concentrations of progestins, higher than those observed in serum, are achieved in selected target tissues, such as in fatty tissue. Thus although all steroid receptors generally appear to function by similar mechanisms, the progestin-specific intracellular actions on target genes via steroid receptors may vary depending of many factors such as those discussed above.

3.3. Effects of old vs. new synthetic progestins on target genes via the:

3.3.1. Progesterone receptor (PR)

The physiological response of a cell to Prog is mediated by the PR, which is expressed in the female reproductive tract, mammary gland, brain, as well as the pituitary gland [131,132]. Prog plays a central role in various reproductive events associated with establishment and maintenance of pregnancy, normal mammary gland development and sexual behaviour [133,134]. PR-mediated responses of Prog differ depending on the target tissue. For example, in the uterus its actions are anti-proliferative, while in the breast it can both proliferate and differentiate [135]. The concentration of endogenous Prog in serum of premenopausal women is low during the follicular phase (0.65 nM) but rises to about 80 nM during the luteal phase, and to about 600 nM during pregnancy [8,136]. Although Prog circulating in blood is bound to corticosteroid binding globulin (CBG) with high affinity and low capacity, it is rapidly metabolised [8,136], with a half-life in serum of only 5 min [8].

The PR acts on promoters containing PRE’s, such as those found in the c-myc gene [137], or by tethering to other transcription factors to regulate promoters lacking PRE’s, such as that of the...
p21(WAF1) cyclin-dependent kinase inhibitor gene [138]. Alternatively, the PR can regulate transcription via non-genomic pathways [105,139]. The complexity of PR-mediated biological responses of Prog is extensive, since Prog can activate two functional isoforms of the PR, PR-A and PR-B, which are transcribed from two promoters of a single gene [140]. PR-B contains an additional sequence of amino acids at its amino terminus, which encodes a third transcription function domain that is absent from PR-A [141,142]. This domain allows binding of coactivators to PR-B, but not to PR-A [143]. Not only do the ratios of the individual isoforms vary in reproductive tissues [144], but they also have different physiological functions in various target tissues including the ovary, breast and uterus [145]. Furthermore, PR-A and PR-B show distinct properties for transactivation that are cell- and promoter-specific [133]. Considering these differences in transcriptional activities between PR-A and PR-B, it is possible that they mediate different physiological responses to Prog, and also to other PR ligands.

All synthetic progestins have been shown to bind to the rat, rabbit or human PR in a number of studies, with most showing a higher relative binding affinity than Prog [59,60,146–154]. Notably, binding affinity differs depending on the species, type of tissue or cell-line, and experimental conditions such as intact vs. cytosolic fractions, highlighting the need for caution when interpreting these results. For example, Bergink and co-workers observed that MPA had a higher relative binding affinity for the PR than NET in intact MCF-7 cells, while MPA and NET had similar relative binding affinities in cytosolic fractions of the same cell line [146]. As illustrated in Table 1, the ranking order in terms of binding affinity for the human PR is GES > TMC > LNG > MPA > NET > Prog [55,60]. In contrast, DGN and DRSP have relatively low binding affinities for the human PR [11] and references therein; [153]. It is unclear from the data in the literature whether the binding activity of these progestins for the PR was determined for the PR-A and/or PR-B isoforms, and whether the number of binding data are Kd or Kc values or RBA (EC50) values.

Considering that PR-A and PR-B can respond to Prog to regulate both overlapping and distinct transcriptional activity that are promoter- and cell-specific [135], and that synthetic progestins can be derivatives of Prog or testosterone or even spironolactone, it is likely that they may have different binding affinities for the PR-A and PR-B isoforms and display different biological activities depending on the target cell. For example, Schoonen and co-workers showed that LNG and GES displayed different binding affinities for PR-A and PR-B [155]. The observation of differential binding affinity, even though the ligand binding domain sequences of PR-A and PR-B are identical, may be explained by the fact that different ligands can induce different conformational changes in the PR-isoforms [156]. It has been shown that Prog and MPA have similar transcriptional activity through both PR-isoforms, via a synthetic PRE2-luciferase construct, as well as on endogenous PR-regulated genes in human breast cancer cell lines expressing either PR-A or PR-B, or both [157]. However it is not known whether other progestins would exert similar activity via the PR isoforms in these cells, and whether these responses would be cell-specific.

Although the contraceptive effect, as well as the anti-proliferative effect on the endometrium in HRT is well-documented, the intracellular mechanisms and the genes targeted by Prog and progestins for contraception and HRT via the PR are not easily found in the literature. However, the progestins have been shown to be PR agonists for transactivation via synthetic PRE sequences as well as via natural promoter constructs for several target genes involved in other physiological processes such as tumour development, immune- and cardiovascular function [43,158–160]. When investigating the effect of progestins in the pathogenesis of endometriosis, results suggest that MPA has opposite effects to those of Prog, such as the finding that MPA represses expression of the chemokine RANTES (Regulated-upon-Activation, Normal T cell Expressed and Secreted) gene via the PR in cultured human endometrial stromal cells [161], while the expression of RANTES significantly increased in Prog–exposed primary endometrial T cells [162]. In addition, MPA, NET-A and LNG were shown to dose-dependently increase expression of two markers of vascular inflammation, intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), in part via the PR [163]. In contrast, Prog and DNG did not affect ICAM-1 or VCAM-1 expression, suggesting that Prog and DNG, unlike MPA, NET-A and LNG, do not stimulate cell adhesion molecules and would thus prevent atherosclerosis in postmenopausal women using a regimen of estrogen combined with either Prog or DNG. Similarly, a study in isolated human endothelial cells as well as in ovariectomized rat aortas, demonstrated that MPA has no effect on endothelial nitric oxide (NO) production, while Prog increases its production [164]. The authors also showed that DRSP, like Prog, increases the synthesis of NO, via the PR [165]. Since production of NO, a vasodilatory agent, is essential for normal vascular endothelial functioning, impaired synthesis of NO would most likely result in atherosclerosis [166]. Thus, these studies imply that Prog and DRSP would have beneficial effects on the vasculature, while MPA would not. Of note, this data, as well as the reported biological activities in Table 2, indicate that MPA, NET-A and LNG, unlike DNG and DRSP, do not mimic the actions of natural Prog.

MPA has been shown to increase vascular endothelial growth factor (VEGF) promoter construct activity in Ishikawa endometrial adenocarcinoma cells co-transfected with either hPRA or hPRB [167]. Similarly, NET also increased VEGF release into the media of cultured T47D breast cancer cells [158], an effect likely mediated via the three functional PRE elements located in the promoter [167]. These results raise the possibility that increased angiogenesis in response to MPA, and possibly other progestins, may play a role in cell growth or metastasis in some human tumours [158]. However, the role of progestins in cancer development is controversial. MPA induces human breast cancer cell proliferation by increasing cyclin D1 promoter activity via PR-B, but not PR-A [160]. As the cyclin D1 promoter does not contain PRE-related sequences, this effect appears to be mediated via a non-genomic mechanism which entails activation of the PI3K/Akt/NFkB signaling cascade [160]. In support of the non-genomic activation of cyclin D1 by PR-B, a recent study showed that the progestin R5020 induces cyclin D1 via PR-B by a mechanism dependent on a direct interaction between the SH3 domain interaction motif in PR-B with the SH3 domain in SRC, thereby activating the SRC/MAPK signaling pathway [168]. In contrast, MPA, like Prog, effectively inhibited estrogen-induced growth of the T47D cell line, and also inhibited growth of the Ishikawa endometrial cancer cell line via stably transfected PR-B, but not PR-A, in the absence or presence of estrogen, suggesting both positive and negative effects by MPA in breast cancer. Prog, MPA and DRSP, alone or in combination with E2, have also been implicated in increased breast cancer cell migration via the PR by a mechanism involving the activation of the actin-binding protein moesin, and the initiation of actin remodelling [169]. Interestingly, the progestins displayed differences in potency, with MPA being the most potent and DRSP the least. The authors suggest that these compounds may play a role in the development of PR+ breast cancer by changing the ability of cancer cells to interact with the extracellular environment, and ultimately their ability to move or invade the surrounding environment. As DRSP is less potent than MPA, as well as Prog, it may imply that women using DRSP may have decreased PR+ breast cancer than those using MPA.

Consistent with decreased effects on breast cancer with DRSP vs. MPA, a study using expression arrays demonstrated that DRSP displayed very weak effects on the transcriptional profile of PR-regulated gene expression in the PR-positive T47Dco breast cancer
cell line, when compared to Prog, MPA, NET-A, LNG and TMG [159]. Notably, Prog and the other progestins showed similar effects. Similarly, a recent study showed that Prog, MPA and NET are similar in terms of agonist potency for transactivation, while DRSP displays a much lower potency [39]. Taken together, these results indicate that natural Prog, MPA, NET-A, LNG and TMG show a high degree of similarity in their PR-mediated transcriptional responses, while DRSP and LNG display weaker transcriptional effects.

Although the above studies shed some light on the mechanism of action of Prog and synthetic progestins via the PR, on physiological functions such as breast cancer, it is clear that we are nowhere close to understanding the complexity of downstream effects of these ligands binding to PR-A and/or PR-B in different Prog target tissues. In addition, very little is known on binding of progestins to PRs located on the membrane of several cells. It is probable that some of the effects of progestins on the central nervous system, for example, are mediated by these membrane-receptors.

3.3.2. Glucocorticoid receptor (GR)

Although the GR is ubiquitously expressed, its levels are regulated in a tissue- and cell cycle-specific manner [79]. In addition, there are several different isoforms of the GR, such as GRα and GRβ, which exhibit differential expression profiles and functions [170]. GR ligands, called glucocorticoids (GCs), play a crucial role in numerous physiological functions, including the functioning of the central nervous system (CNS), digestive, hematopoietic, renal, and reproductive system. GCs can also alter immune responses by regulating the activity of leukocytes and by the suppression of cytokine and chemokine production [79]. Prog binds the GR with low relative affinity and displays weak partial agonist activity for transactivation and transrepression via the GR [152,171]. Consistent with this, some biological assays in rats suggest that Prog displays GC activity, while some report no GC activity in vitro (Table 2). It appears to be generally accepted that the ideal synthetic progestin should display no activity via the GR [56].

However, a large number of studies have shown that MPA has a high relative binding affinity for the GR [43,45,111,171–173], although the affinities determined in different studies vary (Table 1). Interestingly, a study in human mononuclear leukocytes found that MPA displayed significantly higher binding affinity towards the GR than cortisol, the endogenous GC in humans [173]. The third generation progestin, GES, was shown in some studies to also bind with a relatively high affinity to the GR [60,174]. Interestingly, a study in human mononuclear leukocytes found that MPA displayed significantly higher binding affinity towards the GR than cortisol, the endogenous GC in humans [173]. The third generation progestin, GES, was shown in some studies to also bind with a relatively high affinity to the GR [60,174]. (Table 1). In contrast to MPA and GES, NET, LNG, DNG, and TMG, like Prog, have been shown to bind the GR with high relative affinity [11,60,111,153,171,173,175] and references therein; [38] (Table 1). Whether MPA is likely to exert GC activity in vivo in the presence of cortisol in humans, is unclear. The affinity of MPA is about twice that of cortisol for the GR [173], while only about 4% of cortisol is free, since most remains bound to CBG. In contrast, MPA does not bind to sex hormone binding globulin (SHBG) or CBG [11,39]. MPA used as injectable contraceptive, exhibits peak concentrations of about 65 nM [117] in serum, while free cortisol levels average about 11 nM daily, but peak at about 19 nM in the morning and drop to about 3 nM at night [176]. Taken together, it is thus likely that MPA does compete with cortisol for binding to the GR under some conditions and in some tissues in vivo. There is indeed some evidence for GC activity of MPA in rat models (Table 2), consistent with a GR-mediated effect. In addition, MPA used together with estrogen, has been shown to down regulate release of the pro-inflammatory cytokines interleukin-2 (IL-2) and interferon gamma (IFN-γ) by phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) isolated from postmenopausal women using HRT therapy [30], suggesting a GR role.

Extensive evidence exists that both MPA and Prog, but not NET, exert partial glucocorticoid agonist activity via the GR in cell lines in vitro. Since many of the side-effects of conventional GCs used to treat inflammatory or autoimmune diseases may be attributed to transactivation of GRE-driven promoters [177,178], an important goal of pharmacological and clinical research has been to identify ligands that discriminate between transactivation and transrepression (Fig. 2). The effect of Prog and MPA on transrepression via the GR has previously been investigated in human lymphocytes [43]. Bamberger et al. indicated that MPA acts as a dissociated GC, since it efficiently transrepresses IL-2 transcription, but only minimally activates transcription via GREs, while Prog could not transrepress IL-2 or activate transcription via GREs [43]. In contrast to the minimal transcriptional activity observed for MPA via GREs in human lymphocytes, in our study in HEK293 and L929A cells, that showed MPA displays potent transcriptional activity of a GRE-driven promoter-reporter construct. We also observed significant MPA-induced repression of IL-6 and IL-8 promoter-reporter constructs in L929A cells, which was suggested to be mediated via the GR [45]. Consistent with this, a GR-mediated decrease in IL-6 mRNA expression by MPA has been observed in a human thyroid cancer (KTC-2) cells [44]. We showed that Prog has partial agonist activity for transactivation and transrepression, and that NET-A does not transactivate, while only marginally transrepressing an IL-8 reporter construct. Similar results for Prog, MPA and NET-A were observed in a COS-1 cell system, transiently transfected with hGR and a GRE-driven reporter construct or an AP-1 or NKB promoter–reporter construct [111]. Interestingly, we also showed that, under these conditions, high concentrations of NET-A, but not MPA, display weak GR-mediated antagonist activity (unpublished observations). Whether any of the other progestins display transrepression via the GR is unknown. Consistent with their reported biological activities in animal and tissue culture models (Table 2), DNG, DRSP and TMG, do not possess GC activity via overexpressed GR and synthetic GRE-containing promoters [39,55,152]. Of note, the finding that GR levels can determine whether MPA acts as a GR agonist or antagonist in some systems, and can also influence its dissociative properties (extent of transactivation vs. transrepression activity), could have major implications for the physiological effects of MPA, and maybe even other progestins, via the GR [115].

In addition to the above-mentioned studies, several others indicate that the GR may play a role in the effects of MPA on the immune system. For example, MPA, unlike NET, has been shown to induce GC-like effects including inhibition of the proliferative responses to the T-cell mitogens concanavalin A and phytohaemagglutinin [173]. Since GCs impact on virtually every aspect of the immune and inflammatory response [179,180], MPA may exert side-effects due to it GC-like actions on target genes involved in immune function, other than those reviewed above.

It has been suggested that MPA, but not Prog, can exert negative effects in kidney cells via the GR. For example, MPA, but not Prog, increased endogenous α-subunit of epithelial Na channel (α-ENaC), as well as serum and GC-regulated kinase 1 (sgk1) mRNA expression via the GR in two mouse cortical collecting duct (CCD) cell lines [181]. Evidence that these effects are GR-mediated was found by the finding that the effect was inhibited by RU486, a GR and PR antagonist, but not by the pure PR antagonist, Org31710. Furthermore, treatment of rat vascular smooth muscle primary cells with MPA and GES, in contrast to NET and LNG, upregulated proteolytically activatable thrombin receptor (PAR-1) expression, which markedly potentiated the vascular procoagulant effects of thrombin, an effect thought to be due to the GC-like activities of MPA and GES [182]. Taken together, these studies indicate that unlike Prog, some synthetic progestins such as MPA, and possibly GES, have GC-like adverse effects on early stages of atherosclerosis, while others such as NET and LNG lacking GC properties, may be protective under certain conditions. Furthermore, our study indicating that MPA protected the GR from partial trypsin digestion in vitro to a
much greater extent than NET-A or Prog at saturating concentrations, suggests that the differences in biological activity of these progestins, and possibly others, are not only due to differences in their affinity for the GR, but also due to the induction of different conformational changes in the liganded-GR [171].

Not all GR-mediated effects of MPA appear to be negative. For example, MPA dose-dependently elevated Nm23-H1 (metastasis suppressor) protein expression in metastatic human breast carcinoma cells via the liganded GR binding to GRES [183]. From a cancer therapeutic perspective, this elevation of the metastasis suppressor expression by MPA is beneficial. Consistent with positive effects, it was recently shown that both Prog and MPA down regulate endothelial nitric oxide synthase (eNOS) mRNA expression and the formation of nitric oxide levels in human umbilical vein endothelial cells (HUVECs) via the GR [184], thereby reducing the anti-aggregatory effect of endothelial cells. Considering that Prog is only a weak partial agonist via the GR, it is surprising that its effects are GR-mediated, and this raises the question whether GES, which also exhibits weak partial agonist activity, would have similar effects. In contrast, LNG, which does not possess partial GC activity, did not induce the down regulation of eNOS mRNA expression [184], indicating that it would have deleterious effects on the vasculature.

In summary, MPA and GES, unlike Prog, NET-A, LNG, DNG, DRSP and TMG, have high affinity for the GR and exhibit potent GC activity (MPA), or weak GC activity (GES) (Tables 1 and 2). The GC-like actions of MPA and GES may cause side-effects in women using these progestins for reproductive therapies via transactivation and transrepression of GC-responsive target genes, and in particular may compromise immune and cardiovascular function. Given that progestins such as NET, LNG, DNG, DRSP and TMG lack GC activity, it is probable that these progestins will not have GC-like adverse effects. Consistent with this idea, MPA displays GC-like negative effects on bone density, while others such as NET and LNG do not [41,42].

3.3.3. Androgen receptor (AR)

The AR is expressed in many different tissues including the mammary gland, muscle, prostate, skin, vagina, bone marrow and testes, with two biologically important isoforms, AR-A and AR-B, known to occur [79]. The AR plays an important role in male reproductive function, as well as in normal ovary and breast development [185,186]. Furthermore, AR knock out mouse models have revealed that AR function is critical for sustaining female fertility [187]. Ligands competing with the natural androgen, dihydrotestosterone (DHT), for binding to the AR, such as some progestins, may thus cause metabolic and physiological side-effects in different tissues in women using these progestins for therapy or intervention. Some adverse effects associated with androgen treatment in women include hirsutism, acne, amenorrhoea, and clitoral enlargement [188].

The biological activity of Prog and synthetic progestins associated with binding to the AR is controversial. Some studies report that Prog binds to the AR with similar relative binding affinity to that of DHT, and displays resulting weak partial agonist activity and weak AR-mediated antagonist activity [39,189] (Africander et al., unpublished). In contrast, it has been reported that Prog binds to the AR with very low affinity (3% vs. 100% for DHT), or does not bind to the AR at all, displaying no androgenic effects, but weak anti-androgenic effects in animal models (reviewed in [128,56]). This antiandrogenic effect is not ascribed to binding to the androgen receptor, but to a competitive inhibition of 5α-reductase activity thereby decreasing the conversion of testosterone to the more active DHT [128]. There does not appear to be clinical evidence for AR-mediated antiandrogenic and anti-androgenic activity of Prog. This raises the question as to whether an ideal progestin should display similar AR-mediated properties to Prog, or whether it should ideally not bind to the AR. The older progestins MPA, NET-A, LNG and GES bind to the AR with high relative affinity, and are agonists, but not antagonists, for the AR [43,146,149,150,172,175,189–192] indicating that progestins from the first, second and third generations do not exhibit the properties of Prog. Certainly it seems that some of the new fourth generation progestins like DRSP, DNG, TMG and NOME were designed with the objective of AR-mediated anti-androgenic effects, while others such as NES were not. DRSP, DNG, and TMG have low relative binding affinity for the AR, no AR-mediated agonist activity, and potent anti-androgenic properties [11,38,60] and references therein, while NOMAc binds the AR and has no androgenic activity but displays partial anti-androgenic activity [56,193,194]. In contrast, NES does not bind the AR [195]. For these progestins, whether the reported androgenic or anti-androgenic activity is mediated via binding of the progestin to the AR, or to 5α-reductase, is unclear.

The rationale for using progestins with anti-androgenic activity in HRT is to counteract the androgen-dominant hormonal status in postmenopausal women as a result of decreased levels of SHBG and estrogen (reviewed in [196]). This causes an increase in the levels of free biologically active androgens, which is associated with adverse metabolic effects, such as decreased levels of high-density lipoprotein cholesterol (HDL-C) and increased levels of low-density lipoprotein cholesterol (LDL-C). Evidence from clinical studies supports this rationale, since it has been shown that anti-androgenic progestins such as cyproterone acetate and DNG are associated with increased levels of HDL-C, SHBG and triglycerides (reviewed in [196]). For contraception, the rationale appears to be mainly in the treatment of disorders like acne, hirsutism and premenstrual dysphoric disorder [9,196].

Numerous studies have investigated AR-mediated transcriptional activity using overexpressed AR and synthetic androgen response elements (AREs). For example, androgenic activity of MPA has been shown in Jurkat T lymphoma cells and HeLa cervical carcinoma cells transiently co-transfected with an AR expression vector and different promoter-reporter constructs [43,157]. Similarly, NET, LNG and GES as well as their 5α-reduced derivatives, have also been shown to have AR-mediated transcriptional activation in the HeLa cervical cell line [197]. Recently, a study indicated that, unlike DNG and Prog, neither MPA nor NET antagonized DHT-mediated transcription via overexpressed AR [39]. Like DNG and Prog, DRSP has also been shown to have AR-mediated anti-androgenic activity, but no AR-mediated agonist activity [54]. Similarly, TMG was shown to have very weak AR-mediated androgenic activity on an adenosine HRE-tk-luciferase reporter, while displaying significant anti-androgenic activity in L929 fibroblast cells that express endogenous AR [59].

Most of the above-mentioned studies were done in vitro. The question remains whether AR-mediated androgenic or anti-androgenic effects also occur in vivo, taking into account the relative serum concentrations of natural DHT and typical doses of synthetic progestins, as well as their affinities for the AR and serum binding proteins like CBG and SHBG. The above will also be dependent on the route of delivery, metabolism, pharmacokinetics, as well as target cell- and tissue-specific factors. For example, both MPA and NET-A are likely to exhibit significant activity via the AR in vivo. MPA, NET-A and DHT have similar binding affinities for the AR (Africander et al., unpublished), but the percentage of free DHT is less than 1% [136], while the percentage free for MPA and NET-A is about 100% and 64%, respectively [11]. This is due to the fact that DHT is mostly all bound to SHBG, while only 36% of NET-A is bound to SHBG. In the light of this information, together with the knowledge of the peak MPA, NET-EN and DHT serum concentrations (65 nM, 59 nM [118] and 0.65 nM [136], respectively), it is likely that both MPA and NET-A/NET-EN can compete with DHT for binding to the AR in vivo. Whether Prog can compete with DHT for...
AR binding in vivo is unclear. The relative affinities of Prog vs. DHT for the AR are controversial, but have been reported by some to be similar [39,189] (Africander et al., unpublished). As discussed previously, the in vivo concentrations of Prog vary dramatically in premenopausal women and Prog has a short half-life in vivo. It would thus appear that significant competition is unlikely to occur in the follicular phase, but may occur in the luteal phase and most likely does occur during pregnancy.

The fact that progestins such as MPA, NET, LNG and GES, bind to the AR with relatively high affinity (Table 1) and exhibit partial agonist activity via the AR in vitro, and androgenic effects in rats (Table 2), suggests that these androgenic effects are most likely AR-mediated. It is not clear whether the anti-androgenic effects in rats of DNG, DRSP and TMG (Table 2) are AR-mediated, since these progestins bind to the AR with relatively low affinity. The role of progestin binding to 5α-reductase in the above effects in vivo is also unclear. Nevertheless, AR-mediated side-effects may occur in several tissues in women using some progestins as contraceptives or in HRT. For example, in the breast, it has been proposed that MPA can disrupt endocrine function by disturbing the normal signaling of natural androgens which play a protective role in breast cancer [198]. Thus MPA acting via the AR may contribute to increased risk of breast cancer in women, as was seen in the ‘Million Women Study’ [15]. This suggests that other progestins with partial agonist activity for the AR may act in a similar manner.

In summary, many of the earlier generation progestins possess androgenic activity, and no anti-androgenic activity in animal models, while most of the new progestins possess anti-androgenic activity and no androgenic activity in these models. The extent to which any of these activities occur via the AR, and mimic those of Prog in vivo in women, is not clear. In addition, the relative advantages of androgenic or anti-androgenic actions of progestins in contraception or HRT are also not clear from the literature. Clinical studies have indicated that anti-androgenic progestins used in HRT, like cyproterone acetate and DNG, are associated with positive metabolic effects and have beneficial effects on skin disorders such as acne, hirsutism and seborrhea (reviewed in [196]). Similarly, the contraceptive use of DRSP combined with EE, has also been shown to be useful in the treatment of mild acne as well as premenstrual dysphoric disorder [9]. In contrast, anti-androgenic activity can cause detrimental effects such as hot flushes, loss of libido, bone loss and osteoporosis [199]. Thus progestins with anti-androgenic activity such as DNG, DRSP and TMG may cause similar side-effects in women.

3.3.4. Mineralocorticoid receptor (MR)

The MR has been shown to be expressed in many tissues, including epithelial tissues such as the kidney and colon [200], as well as nonepithelial cells such as the CNS, the heart, the vasculature and adipocytes [201,202]. Different MR isoforms such as MR-A and MR-B, with differential activity, exist [79]. Aldosterone (Ald), the natural ligand for the MR, is mainly recognized for its action on sodium reabsorption in the distal nephron of the kidney, thereby regulating blood pressure. However, Ald is also implicated in the renal inflammatory processes, and exerts effects on the CNS, blood vessels and the heart. In the cardiovascular system, Ald has hypertrophic and fibrotic effects, and can modify endothelial function. Prog has a similar high affinity for the MR as Ald, displaying weak partial agonist activity for transactivation, but potent antagonist activity for Ald, via the MR [152,203–205]. Although Prog has potent anti-mineralocorticoid properties in vitro, it may only partially antagonize the effects of Ald in vivo at times of high Prog concentrations, such as during the luteal phase of the menstrual cycle and during pregnancy due to the fact that Prog has a short half-life and is rapidly converted to metabolites that do not confer similar anti-mineralocorticoid activity as Prog [206].

The question is raised as to whether an ideal progestin should display similar MR-mediated properties as Prog, or whether it should have no MR interaction. The rationale for design of progestins with anti-mineralocorticoid activity appears to be to prevent cardiovascular complications in postmenopausal women using estrogen/progestin treatment for HRT. Estrogen has salt-retaining and blood pressure raising effects via actions on the renin–angiotensin–aldosterone system (RAAS). Estrogen leads to increased synthesis of angiotensinogen, which subsequently increases aldosterone levels thereby promoting sodium and water retention [207]. Thus estrogen indirectly leads to increases in weight and blood pressure. As Prog is an antagonist of Ald via binding to the MR, it may cause increased sodium excretion and prevent sodium retention, conferring potential blood pressure benefits. However, Rylance et al. observed no changes in blood pressure with Prog administration in normotensive postmenopausal women, but a slight reduction in blood pressure was observed in hypertensive women [208].

Current progestins vary widely in their affinities for the MR (Table 1) and reported anti-mineralocorticoid biological activities (Table 2). In addition, relative affinities reported for a particular progestin are very different, suggesting differences in methodology or cell-specific effects (Table 1). The first generation progestins, MPA and NET-A, like Prog, are both reported to bind to the MR, but the relative affinities from different studies vary widely, with MPA in most cases having a higher affinity for the MR than NET-A (Table 1). However, neither have been shown to exhibit anti-mineralocorticoid activity in rat models (Table 2). In contrast, LNG and GES bind the MR with a relatively high affinity (Table 1) and do exhibit some anti-mineralocorticoid activity in rat models (Table 2).

Unlike MPA and NET-A, the fourth generation progestin, DNG, displays no agonist or antagonist activity for the MR [39] (Table 2). This is in agreement with some previous reports indicating that DNG does not bind to the MR ([11] and references therein) (Table 1). In contrast, the fourth generation progestins DRSP and TMG, were developed to have anti-mineralocorticoid properties with a view to beneficial effects on blood pressure and cardiovascular function [55,58] for HRT usage [209]. Both TMG and DRSP have high RBA’s for the hMR, with DRSP exhibiting an affinity for Ald in a similar range to that of Prog [152,153] (Table 1).

The extent to which progestins exhibit agonist or antagonist activity for transactivation via the MR in vitro and how this relates to the reported in vivo rat activity, is unclear for some progestins. A number of studies report that MPA and NET do not display any agonist or antagonist properties via the MR [55] and references therein, [210]. In contrast, Sasagawa et al. [39] showed that although MPA and NET have no agonist activity via overexpressed MR co-transfected with PRe1-κluc in the COS-1 cell line, both MPA and NET were able to antagonize Ald-mediated transcription via the MR, albeit to a much lesser extent than Prog. The fact that MPA and NET show weak antagonist activity in the study of Sasagawa and co-workers [39], while others do not, may be explained by a previous study indicating that receptor density in a specific cell determines the biological character (agonist or antagonist) of a ligand [115]. In vitro studies confirm that DRSP, like Prog, elicits weak MR agonist activity and is an antagonist via the MR [152,211]. These results are consistent with a mechanism via DRSP binding to the MR to confer the observed anti-mineralocorticoid activity in rats (Table 2).

Given the apparent discrepancies between binding data for the MR and observed anti-mineralocorticoid activity in vivo for some progestins, in particular MPA and NET, the question arises as to whether progestins are likely to compete with Ald for binding to the MR in vivo. Some insight can be obtained by considering the affinity of Ald as compared to MPA and NET for the MR, as well as the bioavailability of these steroids. The plasma levels of Ald in
women is 0.24 nM in the follicular phase of the menstrual cycle and 0.46 nM in the luteal phase [136], with a large percentage of it free as only about 17–22% binds to CBG [136, 212]. Although the binding affinity of Ald for the MR is much higher than that of MPA and NET-A, the serum levels for MPA and NET-EN are more than 100-fold that of Ald, with the percentage free for MPA and NET-A about 100% and 64%, respectively [11]. Thus, it seems possible that MPA and NET-A/NET-EN could compete with Ald for binding to the MR in vivo. Considering the relative affinities of the other progestins for the MR (Table 1), it would also seem possible that those with affinities similar to or higher than MPA, such as DRSP, may compete with Ald for binding to the MR in vivo, provided they are present at high enough free concentrations.

A lack of or very weak anti-mineralocorticoid activity of progestins relative to Prog via the MR, could lead to cardiovascular complications in postmenopausal women using estrogen/progestin treatment for HRT, due to an inability to antagonize the salt-retaining and blood pressure raising effects of estrogens. Biological assays in rats (Table 2) showing no anti-mineralocorticoid activity for MPA, NET and DNG suggest that the usage of these progestins may lead to side-effects such as weight gain, increased blood pressure and subsequent cardiovascular complications. Indeed, Rosano et al. showed that the use of estradiol associated with a high dose of NET-A (10 mg) for HRT, increased blood pressure in hypertensive postmenopausal women [213]. In contrast, the Cochrane comparative review on contraceptive usage of these two progestins reported similar relatively small changes in blood pressure, which are not clinically relevant, for both MPA and NET-EN [12]. However, consistent with positive effects of DRSP on the cardiovascular system, a study in ovariec-tomized female Wistar rats treated with Ald and salt to induce renal injury, showed that estradiol in combination with MPA, but not DRSP, increased the development of kidney injury, sodium retention and increased blood pressure [214]. These results indicate that, in contrast to MPA, DRSP, like Prog, has favourable effects on the cardiovascular system. Also supporting a positive role for anti-MR effects of DRSP, an in vitro study by Seeger et al. in human female aortic endothelial cells showed that, similar to Prog, DRSP, inhibited the Ald-induced upregulation of the adhesion molecule E-selectin, the chemokine monocyte attracting protein-1 (MCP-1), as well as plasminogen activator inhibitor-1 (PAI-1) [211]. The anti-mineralocorticoid effect of DRSP and its potential to decrease water retention and blood pressure has also been demonstrated in a clinical study comparing an oral contraceptive containing 30 mg EE and 3 mg DRSP. The results showed a slight decrease in body weight and blood pressure (reviewed in [215]), consistent with beneficial anti-MR activity for DRSP.

Taken together, the progestins that display anti-mineralocorticoid properties such as DRSP and TMG (Table 2), are likely to cause increased sodium excretion and prevent sodium retention, conferring potential blood pressure and cardiovascular benefits, via their MR activity. Thus DRSP and TMG appear more appealing to use as a component of HRT than MPA, NET-A, LNG or DNG. The need for anti-MR activity for progestins as contraceptives is unclear. Taking into account that the MR has been shown to be expressed in many tissues, off-target unwanted effects of progestins may occur via the MR. Thus, even though DRSP and TMG appear to be the ideal progestins in terms of anti-mineralocorticoid properties, and with steroid receptor selectivity similar to Prog, some adverse MR-mediated effects may still occur. Furthermore, since the anti-mineralocorticoid properties of Prog are diminished as it is rapidly metabolised, it is possible that the longer half-life of some progestins with anti-MR activity may not be beneficial, possibly leading to hypotension. Clearly more research is needed to determine the optimal extent of anti-MR activity for both HRT and contraception, taking into account risk-profiles of users.

3.3.5. Estrogen receptor (ER)

The ER is expressed in a number of tissues including the female reproductive system, brain, lung, and heart (www.nursa.org/10.1621/datasets.02001). It has diverse physiological roles, including those involved in maintenance of the reproductive, cardiovascular, and central nervous systems [216]. Like natural Prog, the progestins LNG, GES, DGN, DRSP and TMG, have been reported to have no binding or transactivation activities via the ER [39, 152] (Tables 1 and 2). One of the requirements for a progestin in HRT is to have “anti-estrogenic” actions in the endometrium. However it should be noted that this does not mean that the progestin binds to the ER and acts as an ER antagonist in the context previously described, but rather refers to it opposing the physiological effects of estrogen on cell proliferation in the endometrium, which can lead to cancer. The mechanism of this “anti-estrogenic” action in the endometrium is via the progestin binding to the PR, which then suppresses ER gene expression, and hence the ability of the cell to respond to estrogen. At the same time, the PR bound to progestins increases the expression of the 17β-hydroxysteroid dehydrogenase (HSD) type 2 enzyme, which inactivates estradiol by converting it to estrone [128]. This is the rationale for not using EE for HRT, since it cannot be inactivated by 17β-HSD type 2. Nevertheless the ER, although not binding to progestins, plays an important role in the actions of progestins, since estrogen-activated ER regulates expression of the PR gene, and hence regulates the response to progestins [128].

However, it is possible that MPA and NET do act via the estrogen receptor, although the evidence is contradictory. Two functional ERs, ESR1 and ESR2, transcribed from different genes, have been identified [85]. Both MPA and NET-A have previously been reported to bind estrogen receptors both in vivo (in the rat uterus) and in vitro [217]. Contrary to these studies, other two studies showed that MPA does not bind to the ER [146, 172], while another study consistent with the former binding results, indicated that it does not have any estrogenic effects [218]. Reports on the estrogenic activity of NET are similarly contradictory. NET has been reported to have no binding or transactivation activities via the ER [146, 175]. However, in other studies NET [218] and its A-ring reduced metabolites [219] have been reported to show intrinsic estrogenic activity. Furthermore, a 5α-reduced metabolite of NET (3β, 5α-NET), has been shown to selectively activate ERα at low concentrations, while ERβ agonistic activity was observed only at high concentrations (1 μM) [220]. Similar results were shown for the 5α-reduced metabolite of GES (3β, 5α-GES) [220] and the 3β, 5α-tetrahydro derivative of LNG [197]. Interestingly, a recent study revealed that NET, but not MPA or Prog, showed preferential agonistic activity towards ERα (EC50 = 39.3 nM), and also minimal agonistic activity towards ERβ (EC50 = 1097.2 nM) [39]. In summary, the differences in the results of the various studies may be due to different extents of metabolism of these progestins in different cells. Thus it may be that MPA and NET themselves do not bind to the respective ER isoforms, but that their metabolites do. This may also be true for GES and LNG, as well as other progestins. However, it is hard to discriminate between the binding of the test compound vs. its metabolites.

4. Conclusions

Progestins are designed to be potent PR agonists like Prog, with better bioavailability and longer half-life. However, the currently available progestins exhibit considerable variation in their binding affinities and in vitro transcriptional effects via other steroid receptors, such as the GR, MR and AR. Thus, they differ extensively in their androgenic, glucocorticoid and mineralocorticoid, as well as...
the respective antagonistic biological activities, in animal models. In addition, many of these reported rat biological activities differ in the literature and need to be further investigated, such as the extent of androgenic, anti-androgenic, anti-mineralocorticoid, and glucocorticoid activity of Prog and progestins. Another confounding factor is that activities of Prog and progestins in rats may not mimic those found in women of appropriate age groups, and hence should be interpreted with caution. Many of the reported progestin activities do not mimic those of Prog, which does have anti-mineralocorticoid, probable anti-androgenic as well as probable glucocorticoid activity in animal models [56]. The optimal properties of a synthetic progestin in terms of activities via the GR, AR and MR, for contraception and HRT, thus appear to be uncertain. This is understandable given the many complex factors that need to be considered, including clinical evidence indicating both positive and negative effects of many of the above off-target activities via steroid receptors. It should also be noted that clinical trials have shown that contraceptives currently used are generally safe and effective, and mostly, the benefits appear to outweigh the risks for those functions assessed. However, it is becoming increasingly evident that the design of progestins effective in contraception or HRT, but with minimal side-effect profiles, is extremely complex, and that a better understanding of mechanisms of intracellular progestin action is required.

Tissue-specific actions of progestins may play an important role in determination of intracellular progestin actions, and be informative regarding predicted beneficial or adverse effects in particular tissues. Given the emerging complexity of steroid receptor signaling, more research is required to better understand the cellular and tissue-specific intracellular responses to progestins, including promoter-specific effects; efficacies and potencies via different steroid receptors for regulation of transcription by both direct DNA-binding as well as tethering mechanisms; non-genomic signaling pathways; the role of intracellular ligand metabolism, and relative concentrations of classical nuclear receptor isoforms and membrane steroid receptors. For example, it is evident that the interpretation of most of the progestin steroid receptor binding data in the literature is not straightforward. Studies determining binding affinities in tissue or cell lines, from human and other mammalian origins, are often confounded by the presence of multiple receptors and their isoforms. It is thus possible that some of the results in the literature may be misleading. To determine accurate $K_d$ values of different ligands, some advantage can be obtained by overexpression in a steroid receptor-deficient cell line such as COS-1 or CV-1 cells. This allows a comparison of the relative binding affinities of ligands for the receptor within a system where only that particular receptor is present. Recent research at the molecular level has begun to address these issues by using suitable model systems to gain more accurate measures of affinity of progestins for specific receptors in the absence of other steroid receptors. Potencies and efficacies for specific target genes relevant to side-effects such as immune function, breast cancer and cardiovascular complications have also been investigated for some progestins, as well as the role of the PR and other receptor isoforms in progestin signaling. Some large scale microarray analyses of gene expression profiles in relevant target cell types have been performed. The role of non-genomic signaling by some progestins in side-effects such as breast cancer has been investigated as well as the role of intracellular progestin metabolism and the role of steroid receptor non-genomic cross talk by some progestins. However most of these studies have been performed with only the older generation progestins. There is still a paucity of published research addressing the relative binding affinity and relative agonist potency and efficacy (relative to each other and to Prog) of MPA, NET-EN (and NET-A), LNG, GES, DNG, DRSP and TMG for transrepression and transactivation via various steroid receptors as well as their effects on intracellular signaling pathways, in the same system. While limited recent research with the newer generation progestins suggest that they possess similar gene expression profiles via the PR, these studies need to be extended to model systems relevant to other side-effects, possibly mediated via receptors other than the PR. Some such studies have been recently reported and the results show in many cases that different progestins display very different biological effects to each other and to Prog, in a variety of model systems relevant to side-effects, most likely by receptors other than the PR. It is probable that some of the effects of progestins on the central nervous system, for example, are mediated by membrane-receptors, and hence more research is needed on the actions of progestins via these membrane receptors.

In addition, research is needed at the molecular level to investigate the consequences of the longer half-life and increased bioavailability of some more recent progestins, as well as their tissue-specific metabolism and possible clinical implications of progestin activity via, for example, different receptor isoforms. One factor hampering such research is the lack of availability of newer generation progestins and receptor-specific antagonists as tools, to the basic researcher. The limited physiological relevance of transformed cell lines used as model systems to measure direct effects of progestins also limits these molecular studies, which could be more relevant in primary cell models. In addition, given the critical dependency of a biological response on progestin concentration, more information about free serum and intracellular concentrations of progestins in women undergoing treatment, would be helpful in understanding the physiological relevance of in vitro experiments. Furthermore, the literature can be confusing since it appears to be common to refer to MPA as "progesterone", suggesting that authors are not always aware of possible differences in the biological effects of a particular synthetic progestin vs. endogenous Prog [221].

The risk factors associated with the use of MPA as an injectable contraceptive and in HRT need more attention. Although MPA appears to be a relatively safe and highly effective, convenient, injectable contraceptive option, it may not be ideal for certain risk groups. MPA has been shown to exhibit no increase in cardiovascular events, breast cancer, other gynaecological malignancies, or postmenopausal fractures in long-term contraceptive users [10,222]. However, while MPA possesses androgenic activity and no anti-mineralocorticoid activity, it does have relatively potent glucocorticoid activity. There would thus seem to be little scientific justification for the continued widespread usage of MPA in HRT. However it is possible that MPA, with both progestational (PR) and anti-inflammatory (GR) activity, could offer some clinical benefits, possibly due to its glucocorticoid-like actions [221,223]. Nevertheless, recent clinical evidence showing that MPA and NET, in combination with estrogen, increase the risk of breast cancer and that MPA increases the risk of cardiovascular complications in long term HRT users [14,15] has raised concerns about choice of progestin for HRT. Furthermore, MPA used as an injectable contraceptive has recently been shown to be associated with increased HIV acquisition [224], adding impetus for increased research into the mechanism of action of progestins, especially MPA. Compared to Prog and other progestins, MPA has the highest glucocorticoid-like activity, and has a higher affinity for the GR than cortisol. Considerations of receptor theory, serum concentrations of MPA and endogenous steroids, relative affinities for steroid receptors and serum binding proteins, as well as in vitro experiments, suggest that MPA is likely to exert partial to full agonistic, or even antagonist effects via the GR in vivo, in a tissue specific manner, dependent on GR concentrations. Further research is needed to investigate effects of varying contraceptive doses of MPA on immune function and susceptibility to infections, as well as effects of MPA usage in HRT, in appropriate and specific tissues and cell types.
While in vitro experiments in cell culture are informative and can aid in the design of synthetic progestins and testing of specific molecular mechanisms, ultimately more large scale clinical trials need to be performed to directly compare beneficial and adverse effects associated with estrogen only HRT, as well as estrogen combined with newer generation progestins such as DROS and TMC. Ideally, besides monitoring side-effects on breast and uterine cancer and cardiovascular function, other side-effects such as on susceptibility, progression and transmission of infectious diseases, cervicovaginal immune function and neurological processes should be monitored. The design of different progestins for use by particular patients with specific risk profiles, based on a thorough understanding of the molecular mechanism of progestin action, would be something to strive for in future.

References

Moore MR, Zhou JL, Blankenship KA, Strobl JS, Edwards DP, Gentry RN. A
Dunn JF, Nisula BC, Rodbard D. Transport of steroid hormones: bind-
Clarke CL. Cell-specific regulation of progesterone receptor in the female
Sitruk-Ware R. Routes of delivery for progesterone and progestins. Maturitas
Odlind V, Weiner E, Johansson ED. Plasma levels of norethindrone and effect
Fotherby K. Variability of pharmacokinetic parameters for contraceptive
Feil PD, Bardin CW. The use of medroxyprogesterone acetate to study pro-
Mulac-Jericevic B, Conneely OM. Reproductive tissue selective actions of pro-
Wen DX, Xu YF, Mais DE, Goldman ME, McDonnell DP. The A and B iso-
Kloosterboer HJ, Vonk-Noordegraaf CA, Turpijn EW. Selectivity in pro-
Wen DX, Xu YF, Mais DE, Goldman ME, McDonnell DP. The A and B iso-
Mula-Pericic B, Connelly OM. Reproductive tissue selective actions of pro-


