Steroids 76 (2011) 636–652

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Review

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

Donita Africander[a], Nicolette Verhoog[b], Janet P. Hapgood[b,*]

[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

ARTICLE INFO

*Article history:*
Received 6 August 2010
Received in revised form 2 March 2011
Accepted 6 March 2011
Available online 15 March 2011

*Keywords:*
Progestins
Steroid receptors
Gene expression
Contraception
HRT

ABSTRACT

Synthetic progestins are used by millions of women as contraceptives and in hormone replacement therapy (HRT), although their molecular mechanisms of action are not well understood. The importance of investigating these mechanisms, as compared to those of progesterone, has been highlighted by clinical evidence showing that medroxyprogesterone acetate (MPA), a first generation progestin, increases the risk of breast cancer and coronary heart disease in HRT users. A diverse range of later generation progestins with varying structures and pharmacological properties is available for therapeutic use and it is becoming clear that different progestins elicit beneficial and adverse effects to different extents. These differences in biological activity are likely to be due to many factors including variations in dose, metabolism, pharmacokinetics, bioavailability, and regulation of, and/or binding, to serum-binding proteins and steroidogenic enzymes. Since the intracellular effects on gene expression and cell signaling of steroids are mediated via intracellular steroid receptors, differential actions via the progesterone and other steroid receptors and their isoforms, are likely to be the major cause of differential intracellular actions of progestins. Since many progestins bind not only to the progesterone receptor, but also to the glucocorticoid, androgen, mineralocorticoid, and possibly the estrogen receptors, it is plausible that synthetic progestins exert therapeutic actions as well as side-effects via some of these receptors. Here we review the molecular mechanisms of intracellular actions of old (MPA, norethisterone, levonorgestrel, gestodene) vs. new (drospirenone, dienogest, trimegestone) generation progestins, via steroid receptors.



**Contents**



## 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,

* 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 antiestrogenic progestins 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 compounds 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-OH progesterone 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 androstane 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). DRSP 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 carbolactone group attached to C-17 [53,58]. DNG is a 19-nortestosterone derivative, but differs from other nortestosterone derivates by its cyonamethyl instead of an ethinyl 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 nomogesterol acetate (NOMAc), both 19-norpregnanes like TMG, were designed to have high selectivity for binding to the PR, with little or no