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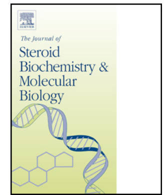

Review

Potency of progestogens used in hormonal therapy: Toward understanding differential actions

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ABSTRACT

Progestogens are widely used in contraception and in hormone therapy. Biochemical and molecular biological evidence suggests that progestogens differ widely in their affinities and transcriptional effects via different steroid receptors, and hence cannot be considered as a single class of compounds. Consistent with these observations, recent clinical evidence suggests that, despite their similar progestogenic actions, these differences underlie different side-effect profiles for cardiovascular disease and susceptibility to infectious diseases. However, choice of progestogen for maximal benefit and minimal side-effects is hampered by insufficient comparative clinical and molecular studies to understand their relative mechanisms of action, as well as their relative potencies for different assays and clinical effects. This review evaluates the usage, meaning and significance of the terms affinity, potency and efficacy in different models systems, with a view to improved understanding of their physiological and pharmacological significance.

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1. Introduction

Progestogens are compounds that exhibit progestational activity, and include both endogenous progesterone (Prog) and synthetic progestogens designed to mimic its actions. A wide variety of synthetic progestogens is available and their common progestogenic effects are exploited for many therapeutic applications in female reproductive medicine, including their use in contraception and for menopausal therapy. However, these synthetic progestogens also exhibit a range of biological effects that differ not only from each other, but also from that of Prog [1–3]. Choice of progestogen for maximal benefit and minimal side-effects is hampered by a limited understanding of their relative mechanisms of action due to insufficient comparative clinical and molecular studies.

Multiple factors such as route of delivery, metabolism and binding to and regulation of serum proteins affect the bioavailability of the active form of progestogens at target cells [2–6]. Progestogens mediate their intracellular effects by modulating transcription of target genes in specific cells via binding not only to the progesterone receptor (PR), but also with varying affinities to other steroid receptors (SRs) such as the glucocorticoid, mineralocorticoid and androgen receptors (GR, MR and AR, respectively) [2,3,7,8].

Abbreviations: SR, steroid receptor; PR, progesterone receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; AR, androgen receptor; ER, estrogen receptor; GREs, glucocorticoid response elements; NFκB, nuclear factor kappa B; AP-1, activator protein-1; RBA, relative binding affinity; Prog, progesterone; MPA, medroxyprogesterone acetate; NOMAC, nomegestrol acetate; R5020, promegestone; TMG, trimegestone; NET, norethisterone/norethindrone; NET, Anorethisterone/norethindrone acetate; DRSP, drospirenone; DHT, dihydrotestosterone; IC_{50}, inhibitor concentration for 50% inhibition.

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It is generally assumed that their progestational effects are mediated via the PR in female reproductive tissue while the plethora of side-effects occur via the GR, AR and MR.

SRs are ligand-activated transcription factors that function by similar genomic mechanisms, but differ in their target genes and tissues [9]. Once the inactive receptor is activated by hormone binding, the hormone receptor–complex translocates to the nucleus where it binds to specific DNA sequences in the promoter regions of target genes to activate (transactivation) gene expression. In contrast, the expression of specific target genes can also be repressed (transrepression) via protein–protein interactions between the receptor and other transcription factors such as nuclear factor-kappa B (NFκB) and activator protein-1 (AP-1) [10].

A number of assays have been developed to elucidate the intracellular mechanisms of action of progestogens via specific receptors. Binding assays are used to determine the affinity of progestogens for a specific receptor in a number of different model systems, including animal or human tissue or cell lines, as well as in vitro systems. In contrast, most of the data on the subsequent relative biological responses via different SRs following binding, including the potency, efficacy, and biocharacter of the progestogens, have been obtained from animal experiments [2,3].

The aim of this review is to evaluate 'potencies' determined by different assays and in different model systems, as well as the meaning and significance of the term 'potency', as applied to progestogens in the current literature.

2. Receptor binding and affinity

The affinity of a progestogen for binding to the PR and other SRs is a major determinant of the potency of its biological response since it affects receptor fractional occupancy and hence the percentage maximal response in a dose response curve. However it should be noted that receptor affinity may not reflect biological activity, which is also affected by the particular conformation of the receptor–ligand complex induced by ligand binding. This is well illustrated by the fact that an antagonist can have a higher affinity for a receptor than an agonist, but exhibits a very different biological response due to the induction of a different receptor conformation as compared to the agonist. Consistent with their different structures, reported affinities of different progestogens for SRs other than the PR vary widely. However, affinities reported for a particular progestogen for a specific SR also vary greatly, most likely due to different methods and sources of biological material used to determine affinity. Table 1 shows a range of relative affinities reported in the literature for progestogens binding to SRs, where the same reference agonist is used for a particular SR, while Supplementary Table 1 shows relative affinities reported using different reference agonists, as well as details of methods and model systems used.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jsbmb.2013.08.001.

Most reported binding data are obtained by performing heterologous competition binding experiments and expressing the results as relative binding affinity (RBA). The constructed binding curves should theoretically be sigmoidal in shape with a Hill slope value of one, for a single ligand binding to one site on a receptor molecule, without cooperative binding. The Hill slope (steep part of the curve) indicates whether cooperative binding occurs, with a slope of one indicating no cooperative binding, while of slope of less or greater than one indicates negative or positive cooperativity, respectively. The IC_{50}, the concentration of unlabeled progestogen (inhibitor or competitor) that corresponds to 50% inhibition of the total specific binding of the radiolabeled reference agonist, can then be determined. Many apparent discrepancies in RBAs reported in the literature are due to the use of different reference ligands (set as 100% RBA). These include promegestone (R5020) versus Prog for the PR, which differ in their RBAs by about five fold, and mibolerone or methyltrienolone (R118) versus testosterone or dihydrotestosterone (DHT) (Supplementary Table 1), where the synthetic agonist have about 100-fold greater RBA than the natural ligands [2,3]. Thus, these apparent RBA differences are not necessarily real differences. RBAs can be directly compared by recalculating values relative to a common ligand, if the information is available.

Another potential source of variable affinities is inherent in the method of RBA determination. While several reports simply use relative IC_{50} values as RBAs [11–15], these are only an approximate measure of relative affinity since IC_{50} varies due to experimental and biological parameters, such as the concentration of radiolabeled steroid and the concentration of the receptor being investigated. Fig. 1A illustrates how the IC_{50} changes in a competition binding assay as a function of receptor concentration. Interestingly, if the Hill slope is not fixed during plotting, it also increases as receptor concentration increases, suggesting that caution should be exerted when interpreting changes in Hill slope in competitive binding studies when receptor concentration is much greater than the true K_d. A more accurate measure of affinity of progestogens for a SR that circumvents these problems can be obtained by saturation binding to obtain an equilibrium dissociation constant (K_d). These K_d values are likely to be more accurate than RBAs determined by competitive binding, provided that the K_d is greater than the total receptor concentration in the assay and that other sources of technical and samples source error are not present. However, only a few reports use saturation binding to measure RBAs of progestogens. For example, K_d values of 10.9 nM and 4.42 nM for the PR have been determined for drospirenone (DRSP), and R5020, respectively [27], while a K_d value of 1.7 nM was determined for medroxyprogesterone acetate (MPA) for the AR [17]. Sometimes RBAs are calculated from K_d values. For example, K_d values were determined for NOMAC and Org2058 for the PR by saturation binding in rat uterus (K_d = 5 nM and 0.6 nM, respectively) and human T47D breast cancer cells (K_d = 4 nM and 3 nM, respectively). RBAs were then calculated relative to Prog set with a RBA of 100%, such that NOMAC and Org2058 displayed RBAs of 67% and 692%, respectively for the PR in rat uterus, and 192% and 212%, respectively for the PR in human T47D cells (Table 1 and Supplementary Table 1). The differences in the K_d values for Org2058 and the RBAs for both ligands relative to Prog most likely reflect different off-target and/or metabolism and/or species effects in the two systems.As an alternative to saturation binding, homologous or heterologous competition binding displacement assays can be used to determine accurate K_d or K_i values using the Cheng–Prussof equation (Supplementary Table 1), provided the concentration of radiolabeled ligand is less than the IC_{50} [19,20]. The K_i is the equilibrium dissociation constant of the unlabeled competitor or inhibitor, and is a true constant that does not vary with receptor concentration in the assay, provided certain experimental restrictions are adhered to. Using this method, similar K_i values were obtained by two groups for Prog and MPA binding to the GR (K_i: 95.2 nM and 215 nM and K_i: 3.7 nM and 10.8 nM, respectively) [21,22]. RBAs for the GR can also be calculated from K_i values (e.g. relative to dexamethasone set at 100%, RBAs for Prog: 0.84%, and 2%, RBAs for MPA: 21.6% and 39%) (Table 1 and Supplementary Table 1). However, when comparing K_i values and RBAs calculated from K_i values obtained from different groups, large discrepancies are still often found. For example, for binding to the AR, a two-fold difference in the RBA of MPA has been reported, while a five- to nine-fold difference in the K_i values has been reported (151%, K_i = 19.4 nM [23]; 75%, K_d = 1.7–3.6 nM [24], RBAs relative to DHT set at 100%), (Table 1 and Supplementary

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