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REVIEW

Competitive Agonists and Antagonists of Steroid Nuclear Receptors: Evolution of the Concept or Its Reversal

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Abstract—The mechanisms displaying pure and mixed steroid agonist/antagonist activity as well as principles underlying *in vivo* action of selective steroid receptor modulators dependent on tissue or cell type including interaction with various types of nuclear receptors are analyzed in this work. Mechanisms of *in vitro* action for mixed agonist/antagonist steroids are discussed depending on: specific features of their interaction with receptor hormone-binding pocket; steroid-dependent allosteric modulation of interaction between hormone–receptor complex and hormone response DNA elements; features of interacting hormone–receptor complex with protein transcriptional coregulators; level and tissue-specific composition of transcriptional coregulators. A novel understanding regarding context-selective modulators replacing the concept of steroid agonists and antagonists is discussed.

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Key words: pure agonists and antagonists, mixed agonists/antagonists, steroid nuclear receptors, steroid-sensitive DNA elements, transcriptional coregulators

Competitive agonists and antagonists of steroids have been long known and widely used in clinical practice. Pure agonists or antagonists are compounds exhibiting no reverse properties in any test systems. They may have various receptor affinities and induce effects of different intensity. It is assumed that the degree of influence of competing compounds on biological parameter is determined by their receptor affinity. Usually, EC_{50} (half-maximal effective concentration) is used to evaluate the activity of pure agonist and antagonist displaying partial agonist activity. It is considered that EC_{50} is proportional to the ligand–receptor binding affinity. Moreover, the maximum effect is also considered as an estimate, which is an ultimate response of a certain biological system (tissue, cell, or gene) to a ligand. Antagonist activity is estimated by using IC_{50} (half-maximal inhibitory concentration) [1-3].

Today, it becomes clearer that the maximum system response, EC_{50}, and IC_{50} for one ligand can markedly differ depending on cell type or tissue *in vivo* as well as gene and expression system *in vitro*. This allows creating better fine-tuned classification of competitive compounds. The first version of such classification was based on introducing ligands of steroid nuclear receptors exhibiting agonist or antagonist activity depending on tissue or cell type, later called selective steroid receptor modulators (SSRMs). Tamoxifen as one of the first discovered and the best known representative of SSRMs is a selective estrogen receptor modulator (SERM) that antagonizes them in mammary gland and acts as an agonist in bone tissue, uterus, cardiovascular system, and HepG2 cells [4, 5]. Discovery of SSRMs resulted in dividing competitive compounds into pure agonists or antagonists and mixed agonists/antagonists (i.e. compounds displaying either agonist or antagonist activity under different conditions); the latter sometimes are called incomplete or partial agonists or antagonists, although such terms are also used in the case when pure agonists or antagonists do not fully reveal their maximum biological effect *in vivo*, which creates some confusion [6-8].

Abbreviations: AF1, 2, 3, activation function 1, 2, 3; BF1, 2, 3, binding function 1, 2, 3; CBI, coactivator binding inhibitor; COSMO, context selective modulator; CTE, *C*-terminal extension; DAC, deacylcortivazol; DBD, DNA-binding domain; EC_{50}, half-maximal effective concentration; ER, estrogen receptor; GR, glucocorticoid receptor; IC_{50}, half-maximal inhibitory concentration; LBD, ligand-binding domain; NRAM, nuclear receptor alternate-site modulator; NTD, *N*-terminal domain; PAA, partial agonist activity; PR, progestin receptor; SERM, selective estrogen receptor modulator; SRE, steroid response element; SSRM, selective steroid receptor modulator; TA_{max}, maximum transcriptional activity; TIF2, transcription intermediary factor-2.

In addition, an attempt to classify competitive antagonists based on present/absent ability to change receptor conformation that creates a permissive environment for its binding to DNA has been made. It turned out that there are antagonists that hinder agonist binding but induce inactive state of a receptor by modifying interaction with heat-shock proteins and interfering with its nuclear translocation or further interaction of hormone–receptor complex with DNA, likely due to the lack of substantial changes in loop conformation between helix 1 and 3 of the receptor, thus preventing its homodimerization. Such antagonists became known as passive antagonists [9-12]. The progestin onapristone is one passive antagonist that does not allow the hormone–receptor complex to bind to DNA. Later, it turned out that steroid antagonists rarely exhibit such mode of action; moreover, it was found that the mode of action for passive antagonists at saturating concentrations changes, and it could be similar to the one of the active antagonists [9, 13]. Active antagonists are much more common, which bind to receptor and alter its activity, stimulate nuclear translocation, and initiate binding of the complex to steroid response elements (SRE) within regulatory regions of target genes by residing in areas necessary for action of agonist–receptor complex, thus interfering with its activity. In this case, it results in not only competition of agonist and antagonist for the receptor-binding pocket, but also competition of antagonist–receptor complex with agonist–receptor complex for binding to SRE, i.e. steroid also acts as an allosteric regulator controlling interaction between hormone–receptor complex and DNA. This mode of action implies that active antagonists can exhibit partial agonist activity (PAA) determined as percentage out of the maximum transcriptional activity (TA_{max}) for reference pure agonist, and represent a group of mixed agonists/antagonists [10-12].

By keeping up with the above definitions for pure agonists and antagonists as well as mixed agonists/antagonists, we attempt to analyze the mechanisms underlying manifestation of mixed steroid agonist/antagonist activity: (i) depending on cell type and target tissue *in vivo* as well as interaction with various types of nuclear receptors; (ii) depending on *in vitro* (a) traits of steroids interacting with hormone-binding receptor pocket, (b) interaction of hormone–receptor complexes with SRE DNA allosterically modulated by steroids, (c) features of interaction between hormone–receptor complex and protein transcriptional coregulators, and (d) level and tissue-specific composition of transcriptional coregulators.

STRUCTURE AND MECHANISM OF ACTIVATION OF STEROID NUCLEAR RECEPTORS

The nuclear receptor superfamily has modular structure that contains functionally different domains: *N*-terminal domain (NTD), DNA-binding domain (DBD), hinge domain, ligand-binding domain (LBD), and in some cases – the additional F domain. Zinc finger DBDs stabilize secondary and tertiary structure of nuclear receptor–DNA complexes. In the absence of ligand, steroid receptors make complexes with heat-shock proteins such as hsp90, etc., wherein their binding site partially overlaps with the site for binding transcriptional coregulators. X-Ray structural analysis data demonstrate that the NTDs of steroid receptors do not form unique 3D-structure and are proteins with disordered indeterminate domain structure (ID-domains) that exists in a form of dynamic conformation sets able to make either ordered or disordered structures depending on certa*in* situations, e.g. ligand binding [14, 15]. The NTD contains the activation function-1 domain (AF1), which can act independently of ligand and resides within the ID-domain. The activation function-2 domain (AF2) is localized in the LBD and becomes activated by ligand due to changes in orientation of its helix-12. Progesterone receptor B contains an additional activation function AF3 at the NTD *N*-terminus that acts synergistically with AF1 and AF2 [5, 11, 16-18].

Steroid–receptor complexes bind as homodimers to DNA SRE consensus sequences within the regulatory regions of target genes, which facilitates both DNA binding and recruitment of coregulators. Dimerization can be mediated via DNA-binding domains of receptors, ligand-binding domains, intermolecular interactions between NTD and LBD from two receptor molecules (N/C-interaction) [19]. Dimerization of the majority of steroid receptors bound to agonists is characterized by N/C-interaction due to existing long NTDs and their direct interaction with a hydrophobic groove in the LBD that binds the coactivator molecule. Dimerized receptors bind to SREs. It is known that SREs for steroid receptors are mostly palindromic, and sometimes have direct and inverted repeats separated by spacer nucleotides of various length and composition. Also, single nucleotide variations are observed within the palindromes and direct or inverted repeats in the SREs. Interaction with agonist ligand and SRE alters 3D-orientation in helix-12 as a part of AF2, which allows the receptor to bind to coactivator, causing transcription initiation. Coactivator proteins modify gene transcriptional activity via several mechanisms: by binding to proteins of basal transcription complex; by regulating chromatin decondensation; by modifying phosphorylation of RNA-polymerase II. In some cases, transcription initiation occurs due to protein–protein interactions without receptor–DNA interaction [15, 17, 20, 21].

Thus, a sequential set of the following binding reactions is necessary to induce transcriptional effect: ligand–receptor → dimerization of ligand–receptor complexes → dimerized complex-SRE → DNA-dimerized complex-coactivator → DNA-dimerized complex-

coactivator-protein of basal transcription complex or other protein involved in transcription initiation. Because of all these interactions, dynamic alterations in conformation of binding partners occur. In this case, the ligand of the steroid receptor can serve not only as competitive agonist/antagonist affecting binding to receptor, but also as an individualized unique allosteric modulator for all subsequent stages of intermolecular interactions.

MECHANISMS UNDERLYING EXPRESSION OF MIXED AGONIST/ANTAGONIST STEROID ACTIVITY

Dependence on *in vivo* Cell/Tissue Type

Currently, several genes or nuclear receptor isoforms are known for almost every class of steroids. A mechanism underlying participation of different receptor types in manifestation of mixed agonist/antagonist steroid properties is accounted for by various overlapping specificity of their affinity to ligands, the ability to make homo- and heterodimers consisting of two distinct receptors that differ in terms of type and degree of transcriptional activity, often resulting in opposite biological activity of steroids due to their various ratios present in different body tissues. In particular, during heterodimerization, one of the receptor types/isoforms is known to serve as an antagonist for signals transduced via another receptor, which results in manifestation of agonist or antagonist properties of steroids depending on receptor homodimer/heterodimer ratio in any particular body tissue. Different nuclear receptors for the same steroid often regulate expression of various target genes by binding via different hormone response DNA elements and activating various transcriptional coregulators [22-25]. Together, this underlies the *in vivo* action of selective steroid receptor modulators. Opportunities for steroids to express oppositely directed tissue-specific effects are also determined by differential expression and different level of coactivators and corepressors in various tissues, opportunity for and efficacy of interaction between a ligand and nuclear receptor specific to other steroid, as well as abundance of the plasma membrane receptors for the same steroid in the body tissues [15].

As an example, the participation of estrogen nuclear receptors ERα and ERβ in exhibiting *in vivo* mixed agonist/antagonist properties of synthetic estrogens can be noted. Nuclear receptors ERα and ERβ are the products of different genes, and their isoforms can be exposed on the plasma membrane and accomplish signal transduction via a non-genomic route. ERα transduces antiapoptotic signal due to activation of MAPK and Akt, whereas ERβ stimulates apoptosis via p38 kinase. The expression profile for such receptors depends on the type of tissue: ERα dominates in hepatocytes and hippocampal cells, ERβ in cells of prostate, ovaries, and lungs. They were similarly abundant in the cells of mammary gland, bone tissue, uterus, and a number of other organs. The NTD containing activation function domain AF1 from two receptors had only 17% homology, whereas homology in the ligand-binding domain containing AF2 was 55%. The size of ligand-binding pockets in ERα and ERβ differs as well. Ligand specificity for binding to ERα and ERβ is similar with respect to binding natural estrogens and tamoxifen, whereas phytoestrogens mainly bind to ERβ. Differences in structure of AF1 and AF2 in both receptors result in recruiting chromatin remodeling coactivators and coregulators specific to various receptors as well as modulating expression of various target genes as demonstrated using micro-templates. These two receptors also differ in their ability to regulate transcription in the absence of a cognate ligand. By using micro-templates in a model system with U20S-ERβ cells, it was demonstrated that a ligand is virtually always required for regulating transcription with ERα, whereas ERβ regulates transcription of 453 genes in the absence of ligand, and 258 genes only being bound to ligand. Another group of genes regulated by ERβ consists of genes whose transcription is regulated in the absence of ERβ ligand, but becomes upregulated upon addition of estradiol. The action of ERβ with lacking ligand is mediated via AF2, as its deletion or replacement for ERβ AF2 results in disappearance of the effect. It is known that ERα agonists are responsible for maintaining structure of bone tissue and regulating activity of fat tissue, whereas their heterodimerization with ERβ interferes with manifestation of such effects. ERα mediates proliferative effect of estrogens on breast cells, whereas ERβ exhibits antiproliferative activity partially while it heterodimerizes with ERα. In connection with this, it is believed that ERα promotes breast cancer, wherein ERβ exhibits suppressive effect. At present, agonists specific to each receptor subtype as shown by *in vivo* testing of biological activity have been synthesized [5, 15, 22-29].

Dependence of the effects on types of receptors is exemplified by progesterone receptor isoforms such as PRA and PRB. PRB is a strong activator for transcription of various genes containing progesterone-dependent promoters in different cell types, wherein PRA is inactive. Moreover, while PRA and PRB colocalize, the former acts as a repressor of PRB activity due to formation of heterodimer complexes. Experiments with differentially targeted PRA and PRB knockouts demonstrated that PRA rather than PRB is responsible for ovulation, uterine decidualization, and embryo implantation, whereas PRB is responsible for development of the lobular-alveolar apparatus in the mammary gland during pregnancy. By using two-hybrid analysis and assessing profile of gene expression in T47D cells in response to pure and mixed progesterone agonists/antagonists, it was demonstrated

that each of them caused individualized changes in gene expression profile [7, 10, 30-32]. Thus, progesterone analogs displaying various affinities to different isoforms of progesterone receptor can serve at tissue level as either agonist or antagonist depending on conjoint or separate expression of both receptor isoforms in the cell as well as ratio between homo- and heterodimer isoforms. Moreover, at the body level the ratio between antagonist and agonist properties of any particular progestin would also depend on its binding to androgen-, gluco-, and mineralocorticoid receptors.

In vitro Mechanisms

Features of interactions between ligands and hormone-binding receptor pocket. Some investigators propose to arrange competitive agonists and antagonists based on the sites they use to interact with hormone-binding receptor pocket keeping in mind that, although such sites overlap, they not always coincide. In the mid-1990s, it was demonstrated that 54 amino acid residues deleted within the *C*-terminus of progesterone receptor differently affect receptor affinity to agonistic progesterone (decrease) and antagonistic RU486 (no change), except that while RU486 interacts with the mutant receptor it begins to function as agonist rather than antagonist. Other mutations within the ligand-binding pocket of progesterone receptor, particularly G722C, lower affinity of the receptor to RU486 without affecting its affinity to progesterone. These data were confirmed using monoclonal antibodies against the *C*-terminus of progesterone receptor. It is assumed that *C*-terminus of the full-size nuclear receptor can act as transcriptional repressor, and agonists attaching to the hormone-binding domain closer to its *C*-terminus cancel such repression, whereas antagonists bound to amino acids closer to the *N*-terminus do not have such effect on the full-size receptor. However, if the *C*-terminus was deleted or blocked with antibodies, they displayed such an effect. A mechanism of interaction between the ligand-binding domain of the mixed agonists/antagonists is supposed to differ from the one for both agonists and antagonists, and likely involves interaction with both *N*- and *C*-terminal regions of the hormone-binding pocket [10, 31, 33].

It was demonstrated that the degree of transcriptional activation does not always reflect binding affinity of the ligand to the nuclear receptor. Ligands of progesterone receptor such as RTI 3021-012 and RTI 3021-022 are also antagonists of glucocorticoid receptor, and the lack of correlation between binding affinity and their antagonistic glucocorticoid activity has been described. Experiments with the nuclear receptor PPARγ acting similarly to steroid receptors demonstrated that the strong agonist rosiglitazone had two orders lower affinity compared to the less strong agonist MRL24, but resulted in stronger stabilization of helix AF2/12 [10, 31, 34].

A role of dimerization and interaction of hormone–receptor complexes with SRE DNA. It is believed that the full transcriptional activity of steroid–receptor complex emerges upon synergistic action of AF1 and AF2 resulting from N/C-interaction leading to dimerization. However, these functions can be exhibited independently. *N*-terminal AF-1 can also interact with the DBD that contributes to modulating structural functions of the nuclear receptor [5, 11, 17, 18, 35]. Conjoint or separate action of AF1 and AF2 depends on the gene type, i.e. selectiveness of its promoter region: e.g. genes regulated by dexamethasone–receptor complex are divided into those dependent on AF1 (gene of insulin-binding protein), dependent on AF2 (genes *PGDF*, *SGK*, etc.), and genes dependent on both AF1 and AF2 (*16PK* gene) [15]. Thus, homodimerization is a multi-step event that involves various receptor domains including ID-domains of NTD depending on the nature of the ligand, which can acquire dynamic types of conformation differing in pure and mixed agonists and antagonists.

In the early 1990s, a classification of steroid antagonists was proposed based on investigations that examined the action of antiprogestins in T47D cells and traits of interactions between hormone–receptor complexes with certain SRE DNA as well as intensity of their transcriptional activity [9]. Because *C*-terminal extensions (CTEs) in the DNA-binding domain and NTD including AF1 are dynamic regions of receptor, they adopt individual conformations depending on interaction with certain SRE DNA, which influence selectivity of acting mixed agonists/antagonists. Currently, SREs are considered to be not only the sites for docking of hormone–receptor complexes, but also specific sites for binding hormone-receptor, which induce individual changes in receptor conformation allowing or forbidding interaction with any particular coactivator. It is possible due to incomplete identity of SRE for one type of receptor in various genes (differ by size and nucleotide composition of spacer region as well as single nucleotide variations within palindromes, direct or inverted SRE repeats) [21]. There are evidences showing changes in receptor effector domains occurring after interaction with SRE. Binding of glucocorticoid–receptor complex with SRE induces changes in the secondary structure of its AF1 domain by stabilizing it. It was shown that the AF2 domain of ER interacts with different transcriptional coregulators resulting from binding between this receptor and various SRE sequences. Also, it was found that steroid agonist/antagonist activity depends not only on nucleotide sequence of SRE in particular gene, but also on transcription factors residing in the vicinity of the hormone–receptor complex bound to the SRE [17, 21, 36, 37].

Modulation of interaction site for coactivators. Three surfaces of the nuclear receptor are involved in

activating transcription: AF1, AF2, and dimerization domain. The majority of coactivators for nuclear receptors interact with the AF2 LBD, but some coactivators can interact with the NTD and modulate activity of AF1. Some corepressors can also interact with the NTD. In addition, the *C*-terminal extension (CTE) in DBD can serve as an interaction site for coregulator proteins [15, 17, 21].

In contrast to full agonists inducing dissociation of corepressor and forming a platform for binding receptor to coactivator, mixed agonists/antagonists allow binding of coactivators, but along with this they preserve the ability to bind corepressors as well. For instance, X-ray structural analysis data of progesterone receptor show that depending on the ligand, its helix-12 can adopt a conformation typical for the action of agonists (progesterone), wherein corepressor dissociation and coactivator binding occur, and a conformation typical for action of antagonist (asoprisnil), keeping contact with corepressor SMRT. In the case of mixed antagonist/agonist mifepristone, the position of helix-12 is more flexible, so that it does not induce its sole fixed antagonist conformation, but rather alters dynamic balance in conformation of helix-12 resulting in destabilized agonist conformation [17, 34].

Apart from such steroid-induced allosteric modulation of specificity and parameters of binding between receptor and coactivator, it was also shown that the receptor interaction site binding to coactivator might be directly inhibited. In 2006, Wang et al. [38] reported data about ERβ bound to two molecules of 4-OH-tamoxifen. One of them was bound to the receptor hormone-binding pocket, whereas the other to the groove binding coactivator, which is usually hidden by helix-12. It is assumed that one molecule of 4-OH-tamoxifen can induce secondary inhibition of coactivator binding. Thus, the receptor groove that binds the coactivator is able to bind low molecular weight compounds, i.e. agents acting as coactivator binding inhibitor (CBI). At present, CBIs with similar mode of action include not only 4-OH-tamoxifen, but also some peptides having structural homology with box-2 from coactivator TIF2 (transcription intermediary factor-2) [39].

Moreover, it was found that the maximal transcription activity of a steroid-dependent gene can change in response to changes in ligand structure without affecting parameters of receptor interaction with coactivator, but triggering in the coactivator individual changes in conformation of the docking site of the next agent involved in signal transduction, as it was shown for glucocorticoid receptor and TIF2 [40].

Dependence of direction of ligand effect on concentration of transcriptional coregulator. Although modern selective modulators of steroid receptors displaying mixed agonist/antagonist activity *in vitro* are designed to differentially modulate the interaction site of AF2 with coregulator protein, the mode of the ultimate response in a gene expression system also depends on concentration of coregulators in given cells.

A role for the level of coregulators in the direction of steroid action was quite clearly demonstrated in the case of tamoxifen, which acts as estrogen antagonist in mammary gland by inducing conformational changes in ERα, which substantially lower or cancel its ability to interact with coactivator. However, the impact of such conformational changes can be overcome upon increasing concentration of coactivator in the cell, so that tamoxifen starts to act as an estrogen agonist. Indeed, several evidences confirm this concept. For example, resistance to antiestrogen effects of tamoxifen occurring in breast cancer correlates with upregulated expression of coactivators for ERα such as SRC-1 and SRC-3. A special set of experiments done with yeasts expressing reporter gene demonstrated that during interaction of tamoxifen with ERα, its activity reverses from antagonist to agonist after destruction of corepressor. Further, by incorporating various numbers of expression vectors encoding coactivator SRC-1 or corepressor SMRT into HepG2 cells, where 4-OH-tamoxifen acts as ERα agonist, it was shown that 4-OH-tamoxifen-directed activity can be controlled: expression of exogenous coactivator SRC-1 enhances agonist activity of 4-OH-tamoxifen in a dose-dependent manner, whereas expression of corepressor SMRT abandons its agonist activity without affecting activity of estradiol. By coexpressing this corepressor and coactivator SRC-1, it also inhibited agonist activity of 4-OH-tamoxifen. In similar experiments done by co-transfecting U2OS cells with various concentrations of plasmid containing TIF2 gene together with a constant amount of plasmid bearing the gene encoding glucocorticoid receptor, it was shown that the degree of upregulated TA_{max} and downregulated EC_{50} for inducing expression of luciferase as a reporter protein in transfected cells depended on concentration of coactivator TIF2 in response to dexamethasone [17, 37, 41, 42].

MECHANISMS OF INDEPENDENT REGULATION OF PARAMETERS (TA_{max}, EC_{50}, PAA) OF STEROID TRANSCRIPTIONAL ACTIVITY

Selective modulation of parameters for gene induction and ability to separately alter TA_{max}, EC_{50}, and PAA were found for all steroid–receptor complexes. It is known that the transactivation domain of the AF1 NTD rather than the AF2 LBD is much more involved in regulating TA_{max}. Moreover, specific features of dimerization also regulate it [1]. It was found that TA_{max} and EC_{50} could be independently regulated. The rationale of the experiment was as follows: by using transfecting PBMCs with siRNA targeting endogenous coactivator TIF2, it markedly downregulated TIF2 expression. After that,

cultured cells were added with glucocorticoid followed by evaluating parameters of induced mRNA expression of three different genes (*GILZ*, *CD163*, and *THBS1*). Decreased level of endogenous TIF2 protein differently influenced the examined genes: no parameters significantly changed in the case of CD163, whereas some of the parameters were affected for GILZ and THBS1, so that while TIF2 level was lowered it only altered EC_{50} for THBS1, whereas in GILZ, in contrast, it did not affect EC_{50}, but lowered TA_{max} [1, 40, 43].

Now, the idea that EC_{50} and TA_{max} always change proportionately to the ligand binding affinity has become questioned as well. In particular, by using X-ray structural analysis, two mutations in the ligand-binding pocket were selected, which account for binding of structurally distinct glucocorticoids such as dexamethasone deacylcortivazol (DAC). These mutations weakly affected binding affinity of these steroids to glucocorticoid receptor. However, upon inducing expression of two reporter genes, they resulted in markedly increased EC_{50} that exceeded the predicted level based on changes in binding affinity by 15-90-fold. A ligand-dependent decrease in TA_{max} was also observed, which was more pronounced for dexamethasone [1, 44]. The key data, which resulted in understanding mechanisms underlying rise of EC_{50} in this case, were based on experiments with shortened glucocorticoid receptor lacking the AF1 transactivation domain. This mutation did not change the affinity of TIF2 to steroid–receptor complex, whereas parameters of transcriptional activity such as TA_{max}, EC_{50}, and PAA were regulated by coactivator TIF2, etc., similarly to the full-size receptor [45]. Under these conditions, addition of TIF2 to the shortened glucocorticoid receptor resulted in much greater decrease in EC_{50} for one ligand (DAC) compared to the other (dexamethasone). Thus, modification of tertiary structure of TIF2 bound to the receptor depends on type of ligand, which is more pronounced upon binding of mutant glucocorticoid receptor to dexamethasone rather than to DAC. Similar results were obtained for another transcriptional coregulator, Ubc9, in a similar set of experiments, taking into account that the binding affinity for the two other glucocorticoids was the same as shown by using co-immunoprecipitation [40, 44, 46].

Ability of coregulators to selectively modulate one or more parameters of transcriptional induction depending on ligand suggests that one set of molecular interactions control TA_{max}, and that EC_{50} and PAA are determined by other interactions almost in the same conditions, i.e. modulation of EC_{50} and PAA must require certain receptor surfaces and certain coregulators, which differ from those involved in modulating TA_{max}.

Thus, it is clear from the above that in contrast to the classic model describing ligands acting on steroid receptors according to the principle "switch-on/off", each ligand results in individual changes in the receptor surface by creating ligand-dependent platforms for a series of following protein–protein interactions. In connection with this, a multi-step process such as induction of transcriptional activity has been proposed to be described by a mathematical model for a series of reactions for ligand–receptor formation instead of the Hill equation describing it as a single-step reaction. The model takes into account that complexes are bound in a relatively weak manner, existing only transiently, but preserving biological activity. The model was tested and found to strictly reproduce curves for transcription induction upon various concentrations of endogenous glucocorticoid receptor and coregulator Ubc9 [3, 47-49].

Thus, evolving understanding regarding pure and mixed agonists/antagonists, which interact with LBD of steroid receptors, resulted in coining the new term "context selective modulators" (COSMOs), where "a context" is used in the context of promoter for a certain steroid-dependent gene including structure and location of SREs as well as context of specific coregulators interacting with various surfaces of the receptor after ligand binding and interaction between hormone–receptor complex and DNA [39, 50-53].

At the same time, it becomes clear that agonists and antagonists influencing transcriptional activity of steroid receptors can be developed not only at the level of interaction between ligand and receptor hormone-binding pocket, but also at the level of all following stages inducing transcriptional effects. In connection with this, several crucial points in regulating transcriptional activity of steroid receptor were proposed: (i) binding function 1 (BF1) domain – ligand-binding pocket of LBD that interacts with steroid agonists or antagonists; (ii) binding function 2 (BF2) domain – 6-8 amino acid hydrophobic groove formed within AF2 after binding agonist that attaches coactivators (e.g. SRCs) able to interact with coactivator binding inhibitors (CBIs); (iii) binding function 3 (BF3) domain – earlier unknown site of hydrophobic binding, with size similar to AF2 located nearby conjunction between helix -H1, -H3-H5, and -H9. Moreover, other sites for inhibitory compounds exist in the molecule of nuclear receptors, e.g. dimerization surface and DBD zinc fingers. X-Ray analysis done with complexes of dimerized hormone–receptor and DNA made it possible to determine such sites as well as compounds that interact with them. These compounds were called nuclear receptor alternate-site modulators (NRAMs). Finally, transcriptional activity of steroid–receptor complex can be regulated by compounds interacting with minor groove of consensus sequence within a hormone response DNA element [39, 51, 54-56].

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