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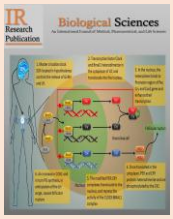

Review of non-genomic actions of sex steroid hormones and devising of algorithm to approach the receptors

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ABSTRACT

Cholesterol-derived hormones also have non-genomic functions. The genomic functions are executed by classical pathways, which are well understood, but non-genomic functions need non-classical pathways. Here is the review of the non-genomic actions along with their non-classical or classical modified receptors along with the pathways of sex steroid hormones and the devising of an algorithm to approach these receptors through an indirect method.

Introduction

Scientists had known the non-genomic effects since 1942 when Hans Selye, for the first time, reported the anesthetic effect of intraperitoneally administered progesterone [1]. The effects of steroid hormones in a short time (within seconds to minutes) and enucleated cells were also reported [2,3]. These observations led to the researchers' intentions to pay heed to the non-genomic functions. Hans Selye, in his publication, set the criteria of non-genomic functions. The classical pathway of steroid actions starts with the entry of steroid hormones due to their lipophilic nature. Steroid Hormones like sex hormones, thyroid hormone (T3), and vitamin D3 have intra- cytoplasmic receptors, while retinoic acid has its receptor in the nucleus. These receptors are transcriptional factors that hasten the gene expression and response shown as protein. Newly synthesized proteins are transported to their effector sites after modifications [4]. These transcriptional factors include the N-terminal domain responsible for ligand binding, a central domain for DNA binding, and a C-terminal domain involved in transactivation. Ligand slightly modifies the receptors by exposing the central domain. This pathway needs 60 to 90 minutes to carry out the genomic function [5].

Any model deviating from the classical pathway is recognized as non-classical. Hans Selye set this criterion [1]. Receptors for non-genomic actions are non-classical and run intracellular signal transduction cascade. There is no direct and initial gene expression. The enucleated cells show non-genomic actions. Nucleated cells do not show gene expression in the presence of dactinomycin, an antibiotic antitumor drug that interferes with the

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DNA uncoiling, and cycloheximide, a protein synthesis inhibitor. The non-genomic effects manifest within a timespan extending from mere seconds to several minutes, but gene expression through indirect pathways takes too long. Some non-classical receptors are on the cell membrane, while others are in the cytoplasm. The term Membrane Initiating Steroid Signaling (MISS) was coined6, but this does not imply that every non-genomic action originates from the membrane of the cell. This study is a comprehensive review encompassing the non-genomic actions of sex steroid hormones and exploring both non-classical and classical modified receptors. The review delves into the intricate pathways associated with these hormones while introducing an innovative algorithmic approach for indirectly targeting these receptors.

Evidence of Receptors that mediate the non-genomic response

It is difficult to prove the presence of receptors directly, so indirect ways are used. These include using agonists and antagonists as classical receptors may mediate to run the non-classical model. Time of response also helps to identify the involvement of non-classical receptors. In cells devoid of DNA or a functional nucleus, steroid hormones exert their effects via non-genomic pathways. The non-genomic pathway exhibits insensitivity to actinomycin D and cycloheximide, as these compounds effectively inhibit transcription [7] and translation [8] processes, respectively. In the cells suspected of the absence of transcriptional factors, the ligand is given in bound form with a polymer that does not let the steroid hormone enter the cell, confirming the presence of the receptor residing on the cell membrane. This review elucidates the algorithm employed to engage with the receptors, as illustrated in Figure 1. Antibodies are also directed against the receptors' proteins to impair their functions, but we know very little about these receptors, so specificity is compromised.

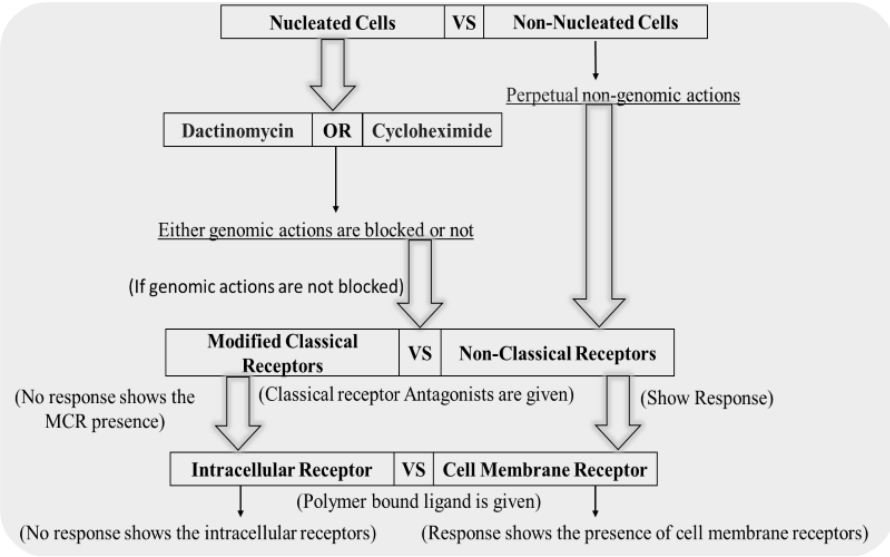

Figure 1. Algorithm to approach the receptors (Indirect Method).

The use of the radioisotope [3H] in the chemical structure of progesterone led to the discovery of progesterone binding protein in porcine liver membranes, which is a direct method to locate the progesterone receptor in the cells. This protein was purified and sequenced, and the DNA sequence was cloned [9]. Subsequently, the results indicated that the progesterone binding sites in porcine liver membranes were associated with the sigma (σ) receptor binding site superfamily, although they might form a distinct subset with a specific affinity for progesterone [10]. In further investigations, the local progesterone-binding membrane protein complex, an oligomer with an approximate molecular mass of around 200 kDa, was identified using antiserum [11]. A research study has provided evidence indicating the participation of carboxyl, tryptophan, and methionine residues in the binding of [3H] progesterone to microsomes from the liver of pigs [12]. The progestin membrane receptor-like components have been exhibited in fish and amphibian oocytes, in human and fish sperm membranes, in bovine ovaries and rat testes, and for progesterone metabolites in breast cancer cells. Candidates for the receptors included nuclear receptors that are membrane-bounded, novel receptor proteins, and receptors with different characteristics from those of any known receptors. Progesterone membrane receptor member 1 (PGMRC1) and membrane progestin receptors (mPRs) are two new receptor families unrelated to any formerly recognized receptor proteins [13]. Current evidence supports that PGRMC1 is thought to also play a role in sterol metabolism, homeostasis, and cell survival [14]. "PGRMC1 was determined to be the sigma-2 receptor (S2R) [15]. According to the unequivocal findings, PGRMC1 is not described initially true S2R [16].

Non-genomic actions of Sex Steroid Hormones

Estrogen

The broad spectrum of physiological functions is modulated by estrogen. High calcium levels at sub-nanomolar concentrations have been reported in

Table 1. Estrogen Receptors with their actions [17–19,22,24,25,27,30–33]

Receptor	Response
GPCRs	Vasodilation, Auditory Functions
Opioid-μ receptor	Increases excitability in arcuate nucleus
GABA receptor	Antagonizes the suppression of neurons
ERα	Cell Progression, Prevent Apoptosis
ERβ	Activation of Caspases-3

Table 2. PRs with their response and pathways [36,39–47]

mPRα	Oocyte Maturation, Sperm hypermotility, Antiapoptotic effect, Inhibit GnRH release	Gi-protein coupled receptors
mPRβ		
mPRγ	Gamete transport and ciliary movement in ciliated epithelium of the fallopian tube	
mPRδ	Non-classical antiapoptotic activity intermediaries of CNS neurosteroids	Gs-protein coupled receptors
mPRε		
PGRMC1	Heme binding, Cell Cycle modulation, Cholesterol synthesis	Serine and Tyrosine phosphorylation

Table 3. Testosterone receptor with their response [48-53, 55,57,61]

Receptors	Response
Gi-PCRs	Vasorelaxation, Muscle Relaxation
LXR	Cholesterol Clearance
β2 adrenergic receptor	Bronchodilation

maturing oocyte and endometrial cells [17,18]. E2 alpha receptors are associated with endothelial nitric oxide synthase (eNOS) via Gi-coupled proteins to induce vasodilation [19], which shows the MISS. Recent findings have unveiled a compelling connection between estrogen and the promotion of gaseous signalling molecules, such as NO, carbon monoxide (CO), and hydrogen sulphide (H2S), which play a pivotal role in modulating cardiovascular function via estrogen's non-genomic mechanisms [20,21]. In pair-fed ovariectomized (OVX) mice, E2 profoundly influences energy metabolism through genomic and non-genomic pathways, which foster leanness [22]. Swift and non-genomic ER signalling is a pivotal factor in orchestrating the tissue-specific attributes of ER modulators. In both in vitro and in vivo investigations, Icariin, a bioactive flavonoid known for its impact on bone health, has been documented for its distinctive capacity to engage non-genomic ERα signalling pathways selectively [23]. It is also reported that estrogen can affect neuronal excitability. Gamma-aminobutyric acid (GABA)-mediated and μ-opioid receptor suppression can be disinhibited by 17-estradiol, which increases excitability in the arcuate nucleus. This is done by lowering hyperpolarization of μ-opioid and GABA-mediated neurons [24]. E2–ERα complex carried out both cell cycle progression and prevention of apoptosis via ERK/MAPK and PI3K/AKT pathways. Complex E2–ERβ, on the other hand, is involved in caspase-3 activation via the phosphorylation of P38/MAPK [25]. The primary functions of endogenous estrogen are carried out through nuclear estrogen receptors, specifically ERα and ERβ, with additional regulation of cell growth and apoptosis occurring through non-genomic cytoplasmic pathways [26].

G-protein coupled estrogen receptor (GPER) has been shown to bind estrogen directly and interact functionally with various cell signalling pathways, including the epidermal growth factor receptor (EGFR) pathway, the notch signalling pathway, and the MAPK pathway [27]. Furthermore, notable findings have demonstrated a substantial correlation between GPER and the advancement of various cancer types [28]. By interacting with their membrane receptors, estrogen can serve as a distinct category of input signals directed toward the MAP kinase signal integrator. It is a cellular rheostat finely tuned to modulate its activity in response to the specific pathways that converge upon it [29]. GPERs are also identified in heart and kidney cells [30]. Chemotherapy resistance is induced in breast cancer cells by non-genomic actions of estrogen on the DNA repair pathways like regulation of ataxia telangiectasia (ATM) and DNA-dependent protein kinase estrogen receptor complex (DNA-PK/ER complex) [31]. GPER30 has also been identified to play a role in the malignant progression of canine mammary cancer [32]. GPERs also make a difference in auditory functions and dysfunctions by modulating of the signaling kinases and calcium influx, which ultimately regulate the downstream transcription factors [33].

Progesterone

Progesterone causes the maturation of oocytes and initiates the acrosomal reaction in sperm cells. Progesterone manifests divergent nongenomic impacts on the contractions of the gravid myometrium, mediated through putative L-type voltage-dependent calcium channels [34]. Progesterone and its receptor PR stimulate the growth and viability of breast tumor cells while promoting the expansion of the breast cancer stem cell (BCSC) population [35]. Classical progesterone receptors are nPRs in two isoforms, PR-A and PR-B [36,37]. mPR plays a role in non-classical signaling [38]. mPRs, like G-protein coupled receptors, have seven transmembrane domains [39]. mPRδ and mPRε are the two subtypes of the mPRs which show a high affinity for progesterone. These receptors belong to Gs-PCRs that potentially intermediate the antiapoptotic actions of neuro-steroids in the CNS [40]. Progesterone checks the proliferation and alters the activation of proliferation-related genes that impart the progesterone an anti-tumorigenic property [41].

PGRMC1 comprises the single membrane-spanning domain. These are located on the membrane of the endoplasmic reticulum and Golgi apparatus [42]. According to one finding, P4 activates the cSrc protein by its PR. The intracellular PR and cSrc can interact bidirectionally to control the function of proteins engaged in glioblastoma migration and invasion [43]. PGRMC1 is an evolutionally preserved multifunctional protein. Intracellular roles, including progesterone control, cholesterol synthesis, heme binding, cell cycle modulation and multiple signalizing pathways, are associated with PGRMC1 [44]. The progesterone acts vascular smooth muscle cells through mPRα, which causes muscle relaxation by decreasing calcium levels in cytoplasm [45]. This is accomplished by downregulating RhoA activity and regulating the functions of sarcoendoplasmic reticulum calcium transport ATPase (SERCA2) and PLB through the Gi, MAPK, and Akt signaling pathways [45].

In normal spermatozoa, progesterone controls the α/β hydrolase domain-containing protein 2 (ABHD2) mediated cAMP-PKA signalling pathway, providing a new target for clinical detection and treatment of infertility [46]. The brain contains many progesterone-effector molecules. The metabolite allopregnanolone is the most studied molecule. These results happen quickly and amp up the inhibitory neurotransmission induced by GABA-ARs. PRs are also found in the brain, where they regulate the production of sexual hormones. mPRs and PGRMC1 are two less-studied effector molecules. Both are found in the brain; mPRs appear to control female reproductive hormone release, while PGRMC1 proteins have no known role [47].

Testosterone

Testosterone stimulates LXR and downstream targets in human macrophages through androgen receptor-dependent pathways, causing cholesterol clearance. This may account for some of the anti-atherogenic properties of testosterone that are commonly seen in clinical settings [48]. Non-genomic signalling potentially encompasses the activation of the G-protein second messenger system, which could lead to elevated intracellular calcium levels, potentially influencing contractile characteristics. Furthermore, it can activate the MAPK signalling pathway and the mammalian target of rapamycin (mTOR) pathway signalling [49]. Progesterone membrane binding in the amygdala of male rats is affected by the testosterone [50]. Testosterone causes vasorelaxation [51] by activating the Gi protein-coupled receptors [52]. Non-genomic effects on corpus cavernosum are also reported [53]. In the corpus cavernosum, testosterone controls functional functions, inhibits phosphodiesterase type 5 (PDE5) expression, and promotes the development of cGMP and NOx53. These findings show that testosterone indirectly contributes to human corpus cavernosum (HCC) relaxation by inhibiting PDE5 and having downstream effects on neuronal nitric oxide synthase (nNOS), endodermal nitric oxide synthase (eNOS), and cGMP [54].

The upregulation of the β2 adrenergic receptor potentiates airway smooth muscle relaxation in pigs by salbutamol [55. This upregulation is done by testosterone [56]. Vasorelaxation is the principal non-genomic testosterone vascular pathway57. Testosterone induces Gi/o protein and PKA activation to vasodilate in the endothelium. PKG activation induces independent endothelium vasodilation. Testosterone has cardiovascular benefits, and testosterone replacement therapy can become a treatment for cardiovascular diseases [52,58,59]. There is compelling evidence in humans and animals that testosterone reduces the QT interval, and that testosterone deficiency is linked to QT prolongation, and thus arrhythmogenesis [60]. The physiological concentrations of testosterone induce the fast membrane association of the adrenergic receptors. The phosphorylation of the MAK and Akt signaling pathways mediates this [61]. Nongenomic calcium-mediated events triggered by testosterone in skeletal muscle cells have been discovered. The non-genomic activity begins at the cell membrane and is triggered by the second messenger calcium and inositol trisphosphate (IP3). Non-genomic testosterone can initiate a more effective contraction by mobilizing calcium from the SR, thus demonstrating an increase in the activation of the Ras/MAP/ERK pathway, resulting in increased

force output or contraction velocity in fast-twitch muscle fibres [61].

Conclusion

In conclusion, studying the non-genomic effects of steroid hormones has revealed rapid cellular responses that operate outside traditional genomic pathways. Non-classical receptors, like G-protein coupled estrogen receptors and membrane progesterone receptors, play crucial roles in these actions. Understanding these mechanisms offers the potential for innovative therapeutic approaches. Further research in this field promises to uncover new insights into hormone function and its implications for human and animal health.

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Conflict of interest

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