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Review

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Non-genomic actions of steroid hormones on the contractility of non-vascular smooth muscles

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Non-standard abbreviations: 5α-DHT: 5α-dihydrotestosterone; 5β-DHT: 5β-dihydrotestosterone; AC: adenylate cyclase; Akt: protein kinase B; cAMP: cyclic adenosine monophosphate; cGR: cytoplasmic glucocorticoid receptor; DHEA: dehydroepiandrosterone; ER: estrogen Receptor; ERK: extracellular signal-regulated kinase; GPCRs: G-protein coupled receptors; GPR30: G-protein-coupled receptor 30; GPRC6A: G-protein coupled receptor class C group 6 member A; IP3: inositol triphosphate; KCl: potassium chloride; L-VDCC: L-voltage dependent Ca^{+2} channels; MAPK: mitogen-activated protein kinase; mARs: membrane androgen receptors; mGRs: G-protein coupled glucocorticoid membrane receptors; MLC 20: myosin light chain-20; mPRs: membrane progesterone receptors; NO: nitric oxide; NR: nuclear receptor; PKA: protein kinase A; PKC: protein kinase C; PLC: phospholipase C; ROCCs: receptor operated Ca^{2+} channels; SERCA: sarcoendoplasmic reticulum calcium ATPase pump; SOCCs: store-operated Ca^{2+} channels; ZIP9: ZRT-and Irt-like Protein 9, 7TM: seven transmembrane domain

Abstract

Steroid hormones play an important role in physiological processes. The classical pathway of steroid actions is mediated by nuclear receptors, which regulate genes to modify biological processes. Non-genomic pathways of steroid actions are also known, mediated by cell membrane-located seven transmembrane domain receptors. Sex steroids and glucocorticoids have several membrane receptors already identified to mediate their rapid actions. However, mineralocorticoids have no identified membrane receptors, although their rapid actions are also measurable. In non-vascular smooth muscles (bronchial, uterine, gastrointestinal, and urinary), the rapid actions of steroids are mediated through the modification of the intracellular Ca^{2+} level by various Ca-channels and the cAMP and IP3 system. The non-genomic action can be converted into a genomic one, suggesting that these distinct pathways may interconnect, resulting in convergence between them.

Sex steroids mostly relax all the non-vascular smooth muscles, except androgens and progesterone, which contract colonic and urinary bladder smooth muscles, respectively. Corticosteroids also induce relaxation in bronchial and uterine tissues, but their actions on gastrointestinal and urinary bladder smooth muscles have not been investigated yet. Bile acids also contribute to the smooth muscle contractility.

Although the therapeutic application of the rapid effects of steroid hormones and their analogues for smooth muscle contractility disorders seems remote, the actions and mechanism discovered so far are promising. Further research is needed to expand our knowledge in this field by using existing experience. One of the greatest challenges is to separate genomic and non-genomic effects, but model molecules are available to start this line of research.

1. Introduction

Steroid hormones play an important role in various physiological processes [1]. The physiological regulation of development, growth, reproduction, and systemic homeostasis is attributed to steroid hormones [2]. Steroids are classified as sex steroids (androgens, estrogens, progestogens), and corticosteroids (mineralocorticoids, glucocorticoids) [3]. Bile acids (cholic acid (CA) and chenodeoxycholic (CDCA) as primary, deoxycholic acid (DCA) and lithocholic acid (LCA) as secondary bile acids [4,5]) have also steroidal structure although they are not considered classically as hormones [4–7].

The classical pathway of steroid actions is mediated by nuclear receptors, which regulate genes to modify biological processes. This genomic action results in the longest time gap between drug administration and the manifestation of the effect [8]. However, non-genomic pathways of steroid actions are also known, mediated by cell membrane-located seven transmembrane domain (7TM) receptors, eliciting effect within a few milliseconds. A wide variety of steroid membrane receptors have been identified (**Table 1**), and additional new receptors may be discovered in the future.

The non-genomic actions of steroids in smooth muscles have been the subject of investigation in various studies, most of which are related to vascular smooth muscles, as summarized in several reviews [18–21]. However, their non-genomic, non-vascular smooth muscle effects also seem important for clinical practice. Therefore, the objective of this review is to compile and review studies on the prompt actions of steroids in non-vascular smooth muscles.

2. Mechanisms of the non-genomic actions of steroids on non-vascular smooth muscles

Although corticosteroids may cause bronchial and renal vasoconstriction [22–24], the rapid action of steroids generally induces smooth muscle relaxation in non-vascular tissues, except some gastrointestinal muscles. The non-genomic steroid effect is usually mediated through a membrane located 7TM receptor and the response occurs in seconds or minutes (less than 30 minutes).

2.1. Seven transmembrane domain receptors

While corticosteroids may elicit bronchial and renal vasoconstriction [22–24], the rapid action of steroids generally induces smooth muscle relaxation in non-vascular tissues.

The non-genomic actions of androgens can be mediated via several membrane androgen receptors (mARs) which belong to the 7TM receptor family [25]. Androgens are capable of activating G-protein-coupled receptor family C group 6 member A (GPRC6A), which is coupled with Gi-, and ZIP9 coupled with G_s-protein, inducing smooth muscle relaxation by the increase in intracellular cyclic adenosine monophosphate (cAMP) [10,26]. The action through mAR or membrane proteins leads to reduced membrane permeability and intracellular level of Ca^{2+} via the blockade of L-voltage dependent Ca^{+2} channels (L-VDCC) [10,19,26,27].

Estrogen is a highly specific ligand for G-protein-coupled receptor 30 (GPR30 or GPER) [10,26,28], which links to Gαs proteins, leading to the activation of adenylate cyclase (AC) enzymes and epidermal growth factors [10], thus resulting in a number of non-genomic

physiological functions [10,28]. Through the membrane-localized estrogen receptor ERα36 [which lacks transcriptional ability], estrogen can stimulate pathways including protein kinase A and C (PKA, PKC) and/or mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK), blocks L-VDDC and activates sarcoendoplasmatic reticulum Ca^{2+}-ATPase (SERCA), thereby inducing non-genomic smooth muscle relaxation [26–28].

The binding of progesterone with membrane progesterone receptors (mPRs) leads to an increase in nitric oxide (NO) or cyclic cAMP levels, decreasing myosin light chain-20 (MLC 20) phosphorylation and inhibiting contractions [29]. However, progesterone can also reduce cAMP levels through myometrial mPRs at the end of pregnancy, which contributes to increased uterine contractions and the induction of birth [30].

Corticosteroids also bind to 7TM glucocorticoid membrane receptors (mGRs) initiating smooth muscle relaxation or contraction activating AC/PKA or phospholipase C/inositol triphosphate (PLC/IP3) pathways, respectively [31,32]. Even membrane-impermeable glucocorticoids increase cAMP levels through the stimulation of $G_{\alpha s}$-protein, which can be ceased by siRNA knockdown of $G_{\alpha s}$-protein, suggesting that they target membrane 7TM receptors. The effect was not altered by GPER antagonists G36 and G15, which indicates that GPER is not involved in their cAMP-elevating action [32].

Mineralocorticoids have no specific 7TM receptors for non-genomic action, although mineralocorticoid receptors were proved to cooperate with several membrane receptors, such as epidermal growth factor and platelet-derived growth factor receptors [12].

2.2. Modulation of intracellular Ca^{2+}

Estrogens and androgens exert non-vascular, non-genomic action via the blockage of L-VDCC and receptor-operated Ca^{2+} channels (ROCCs), and activation of SERCA [28,33]. Testosterone and dehydroepiandrosterone (DHEA) are able to block store-operated Ca^{2+} channels (SOCCs) as well [33,34]. Progesterone reduces intracellular Ca^{2+} via the stimulation of mPRα-dependent signaling pathways, including MAPK and Akt signaling [29]. Corticosteroids (and to a lesser extent mineralocorticoid aldosterone) can reduce intracellular Ca^{2+} levels by stimulating the AC/PKA signaling pathway, which leads to the induction of SERCA and smooth muscle relaxation. On the other hand, corticosteroids may increase the intracellular Ca^{2+} level by activating the PLC/IP3 pathway [31].

2.3. The interplay between genomic and non-genomic mechanisms of steroid action

Increasing evidence suggests that genomic and non-genomic mechanisms of steroid action are not mutually exclusive but exhibit a significant degree of crosstalk and convergence. Besides the physiological effects triggered by direct, non-genomic pathways, these pathways can also lead to genomic effects. Studies have found that nuclear receptors can activate genes through both genomic and non-genomic signaling pathways. This suggests that these distinct pathways may be interconnected, resulting in convergence between them [26]. In prostate cancer cells, proliferative action can be regulated by both androgens and estrogens through a non-genomic mechanism. This regulation occurs through the interaction of these steroids with the kinase Src at the plasma

membrane, which initiates a signaling cascade that involves the activation of the MAPK/ERK pathway [35]. The binding of glucocorticoids to mGR is implied in proapoptotic, immune-modulatory, and metabolic pathways. Remarkably, these pathways are also regulated through the cytoplasmic glucocorticoid receptor (cGR). Thus, glucocorticoids can initiate rapid early priming events, establishing the foundation for the subsequent genomic pathway [36].

In contrast, the activation of non-genomic pathways can be triggered by nuclear receptor (NR)-mediated transcription as well. The expression of specific genes influenced by NRs can subsequently activate non-genomic signaling events. This dynamic interplay between genomic and non-genomic pathways plays a role in various processes such as cellular growth and other related functions [26]. In endometrial cells, estradiol has been observed to induce the overexpression of the L-type calcium channel. This overexpression serves as a potential target for the non-genomic pathway, which is initiated through the genomic pathway [37]. In non-smooth muscle breast cancer cells, progestins upregulate the expression of GPR30, a major receptor for non-genomic steroid actions [38]. This mechanism is also possible in smooth muscle, but it has not yet been proven.

The mechanisms of genomic and non-genomic smooth muscle actions of sex steroids and glucocorticoids are summarized in **Figures 1-2**.

3. Non-genomic actions of sex steroids and corticosteroids on various non-vascular smooth muscles

The complex, non-genomic mechanisms of action of steroid hormones induce relaxation or contraction responses in non-vascular smooth muscle tissues. The bronchial and uterine smooth muscles have been fairly well studied, whereas gastrointestinal and urinary tract muscles have been little studied in this area.

3.1. Sex hormones

3.1.1. Bronchial smooth muscle

Female sex hormones, through their rapid actions, relax both human and rodent bronchial smooth muscles. This non-genomic action either blocks Ca^{2+} channels or activates cAMP production, reducing intracellular Ca^{2+} levels (**Table 2**).

Androgens, including dehydroepiandrosterone (DHEA), testosterone and its metabolites 5α- and 5β-dihydrotestosterone (DHT), elicit a rapid relaxant effect on bronchial smooth muscle. Testosterone, 5α-DHT and 5β-DHT inhibit direct parasympathomimetics- and KCl-induced tracheal contractions through blocking calcium channels and the inositol triphosphate receptor. DHEA also elicits a remarkable relaxing effect on bronchial smooth muscle by blocking Ca^{2+}-channels (**Table 3**).

3.1.2. Uterine smooth muscle

On uterine smooth muscles, male and female sex steroids initiate a non-genomic relaxant effect against contracting agents or spontaneous contractions mostly through reducing and increasing intracellular Ca^{2+} and cAMP, respectively. However, estradiol was found to rapidly increase uterine contractions in rabbits, while the action of progesterone is questionable, as it failed to inhibit pregnant uterine contractions in a rat study but showed an inhibitory effect on human pregnant and non-pregnant uteri. (**Table 4**).

Testosterone, 5 α- and 5 β-DHT and DHEA induce non-genomic uterorelaxant effects via the decrease in intracellular calcium ions both in rat and human tissues. They provide a rapid reversible concentration-dependent suppression for both spontaneous and KCl-induced contractions in human myometrium. 5β-DHT exhibits the strongest inhibitory effect on human tissue. Interestingly, the progesterone-induced non-genomic uterorelaxant effect can be augmented by the presence of androgens in human pregnant tissue [48], which suggests that female and male sex hormones might have different sites of action in non-genomic relaxation (**Table 5**).

3.1.3. Gastrointestinal smooth muscles

In rodents and dogs, female sex hormones induce non-genomic relaxation on gastric, colonic and gallbladder smooth muscles mostly by inhibiting calcium entry mechanisms into the smooth muscle cells (**Table 6**).

The limited number of experimental results suggests that the rapid gastrointestinal actions of female sex hormones are similar to their uterine or bronchial effects and may involve a mechanism (NO) that is mostly characteristic of their vascular action.

The non-genomic effects of androgens on gastrointestinal smooth muscles are not uniform. While the gut (ileal and colonic) smooth muscles are contracted, the gallbladder is relaxed. The activation of different pathways lies in the background of these opposite actions (**Table 7**).

It is known that testosterone can rapidly increase intracellular calcium levels in prostate cancer cells via OREX1 [55]. No data are available on the ileal, colonic or gallbladder expression of this receptor, but a higher expression of OREX1 in the gut might explain the androgen contractile response as opposed to gallbladder relaxation. This assumption must be confirmed by experimental investigations.

3.1.4. Urinary tract smooth muscles

The non-genomic actions of sex steroids in the urinary tract have been poorly investigated. Estradiol and progesterone have different actions on the urinary bladder and probably have opposite effects on the cellular entry of Ca^{2+}. Testosterone is a non-genomic urinary bladder-

relaxing hormone that modifies autonomic neuromuscular junctions and potassium channels in the urinary smooth muscle (**Table 8**).

3.2. Corticosteroids

3.2.1. Bronchial smooth muscles

The use of glucocorticoids as anti-inflammatory and antiasthmatic agents in bronchial asthma is a standard choice among physicians. Their genomic action is crucial in the maintenance of receptor sensitivity to β_2-adrenergic receptor selective compounds [59]. Besides their genomic action, the prompt non-genomic effects of glucocorticoids appear to be interesting and clinically important [60]. Although glucocorticoids have vasoconstrictive effects and decrease airway blood flow through a non-genomic pathway [61,62], they can induce rapid bronchodilation via different mechanisms, although in several cases the mechanism was not defined, only the rapid action was described (**Table 9**).

In rodents, the acute inhalation of glucocorticoid budesonide reduced lung resistance in the ovalbumin-induced model and decreased isometric tension in the histamine-induced model. The bronchodilator effect possibly also involves the classic glucocorticoid receptors, since it can be mimicked or inhibited by PKC activators or inhibitors, respectively [63]. Their action also includes the inhibition of PLC, which rapidly reduces intracellular calcium levels by blocking the IP3 signaling mechanism [67].

In human airways, glucocorticoids such as budesonide, fluticasone, and prednisone significantly elevate cAMP levels. However, their bronchodilator effect is very mild, suggesting that human airways are less sensitive to the non-genomic actions alone. When glucocorticoids were combined with β_2-adrenergic agonists, the elevation of cAMP and the relaxing effect were significant within seconds, suggesting that glucocorticoids may potentiate the β_2-adrenergic agonists-induced bronchodilation in a non-genomic way as well [32]. Interestingly, they also exert an inhibitory effect on the cellular uptake of β_2-adrenergic agonists, which may reduce the synergism between the two pharmacological groups [68,69]. The beclomethasone induced acute relaxation is not dependent on the glucocorticoid receptor or the epithelium, as demonstrated by the absence of blockage in the case of mifepristone pretreatment or epithelium removal, respectively. Instead, it occurs through the rapid activation of the $G_{s\alpha}$–cAMP–PKA pathway, since the bronchodilatory effect was sensitive to the $G_{s\alpha}$ antagonist and the cAMP-dependent PKA inhibitor [70–72]. The immediate clinical effects of inhaled glucocorticoids have also been shown to occur even after a single administration. There is evidence that both the genomic and non-genomic actions of glucocorticoids have significance in the therapy of bronchial asthma, and their non-genomic rapid action has therapeutic value in acute mild asthmatic symptoms [73–75].

Although aldosterone has no non-genomic receptor, its rapid action was proved in human airway cells. Upon aldosterone stimulation, a decrease in intracellular Ca^{2+} was detected, which remained unaffected by pretreatment with genomic pathway blockers (actinomycin D and cycloheximide) but was sensitive to intracellular Ca^{2+} mobilizer and G-protein inhibitors. It is suggested that the

rapid action of aldosterone is mediated through the stimulation of thapsigargin-sensitive Ca^{2+} pumps via a G-protein-coupled PKA signaling pathway [76].

3.2.2. Uterine smooth muscle

Glucocorticoids are routinely used in threatening preterm birth to stimulate the production of fetal surfactant before birth to accelerate lung maturation [77], but their effect on the uterus, especially on contraction, is still questionable. Generally, corticosteroids exert an inhibitory effect on contractility, but deoxycorticosterone displays a concentration-dependent biphasic effect. High concentrations lead to contractility inhibition, whereas low concentrations rather induce contraction. Although clinically a single high dose of glucocorticoids leads to uterine contractions in multiple pregnancies in a 2-day interval [78], preclinical pregnant experiments proved that glucocorticoids could inhibit pregnant uterine contractions in a non-genomic pathway, even the mineralocorticoid aldosterone may elicit a moderate relaxing effect. A complex preclinical experiment demonstrated that the administration of glucocorticoids and mineralocorticoids leads to relaxation in both pregnant and non-pregnant rat uteri in vivo and in vitro, although the relaxing effect of mineralocorticoids was very weak (**Table 10**).

3.2.3. Gastrointestinal smooth muscle

The non-genomic effects of corticosteroids on gastrointestinal contractility have been poorly investigated, and few studies have been carried out on their actions. Aldosterone does not exert a prompt inhibitory effect on colonic motility under basal conditions or even when stimulated with carbachol or an electric field. In the epithelium of mammalian and distal colon, aldosterone and fludrocortisone were found to exert rapid non-genomic effects. These effects include the modulation of PKC activity, increased Ca^{2+} entry through verapamil-sensitive calcium channels, PKCα-mediated influence on Na^{+}-H^{+} exchange activity, and regulation of K^{+} recycling. However, glucocorticoids (hydrocortisone and dexamethasone) do not modify these mechanisms at all. Additionally, it is not known how these epithelial actions may affect colonic contractions because these studies focused only on the ion exchange modifications in the epithelium and did not measure any change in contractile responses (**Table 11**).

3.3. Bile acids

The endocrine effects of bile acids are mediated via the stimulation of the nuclear farnesoid X receptor (FXR) and 7TM receptor TGR5 [4,5,17]. Their rapid, non-genomic smooth muscle relaxant effect is mediated through the TGR5 while FXR mediates the slow genomic effect through the stimulation of the transcription process and the release of fibroblast growth factor 19 (FGF19) which stimulates FGF receptors [17]. TGR5 is mainly located in adipose tissues, liver, pancreas, intestinal and gallbladder smooth muscles [4,5,17],and placenta [83], and it is stimulated selectively by LCA [4].

In the gallbladder, the stimulation of TGR5 induces a cAMP signaling pathway [4,7,17,83] and opens K^+-ATP-dependent channels resulting hyperpolarization to promote gallbladder filling [7]. In the gastric smooth muscle, the stimulation of TGR5 causes relaxation through suppression of the RhoA/Rho kinase pathway [4]. In the airways the secondary bile acids antagonize the muscarinic-3 receptors and cause muscle relaxant effect [6]. In the urinary bladder, bile acids provide a rapid muscle relaxant effect via inhibition of NCX [5].

4. Challenges and opportunities of non-genomic actions of steroid hormones on non-vascular smooth muscles in future clinical use

Although the genomic physiological effects of steroid hormones are well-known and used in clinical practice, their non-genomic actions have received less attention in therapy. One reason is that a single dose of steroids to induce a non-genomic rapid effect does not guarantee that no genomic effect will develop. Therefore, the benefit of rapid action can be converted into a genomic adverse effect. However, the combination of non-genomic and genomic effects of glucocorticoids may be advantageous, e.g., in bronchial asthma or in cases of threatening preterm birth, where both the rapid and the long-term effects have a therapeutic asset.

The separation of genomic and non-genomic steroid actions is difficult but not impossible. Estetrol, a human fetal liver-produced estrogen, activates genomic response via nuclear receptors, but does not initiate a non-genomic effect and is, in fact, a non-genomic response antagonist [84]. The androgen derivative 5β-DHT activates only non-genomic membrane receptors and does not stimulate nuclear receptors directly [85]. The further study and chemical modification of these two compounds may be one of the keys to identifying and controlling what makes the difference between the genomic and non-genomic effects of steroids in the molecular structure. Another unanswered question is the existence of membrane receptors for non-genomic mineralocorticoid action. Although no receptor has been found to mediate the rapid action of mineralocorticoids, they have been shown to have a non-genomic relaxing effect in the uterus and colon epithelium.

The most intensively studied non-genomic effect of steroid hormones in non-vascular smooth muscles is their bronchodilatory action. Glucocorticoids have a long history of use in bronchial asthma, in which the genomic and non-genomic actions occur together and have a combined clinical effect, although their genomic action is stronger. However, the prompt bronchodilator effect of sex steroids (especially androgens) is also promising and might be considered as an acute inhalational alternative for asthmatic attacks. Since inhalational antiasthmatic preparations elicit a local effect in the airways, the risk of secondary genomic adverse effects, either systematically or locally, is extremely low [86].

Sex steroids (except progesterone) and glucocorticoids elicit remarkable rapid relaxation in non-pregnant and pregnant uteri. Glucocorticoids have long been used as medicines to stimulate fetal lung maturation; hence their rapid relaxing action may appear along with the surfactant-producing effect. Uterorelaxant sex steroids might be beneficial with their rapid non-genomic action, although their subsequent genomic effect cannot be excluded. Perhaps 5β-DHT has potential since it has no direct genomic action, so this compound and its analogues can be candidates for a

new branch of tocolytic agents. Further experiments are required to prove the efficacy of this androgen derivative in premature pregnant uterine contractions.

The potential use of non-genomic steroid actions in gastrointestinal motility disorders currently appears to be the one of the most uncertain. Only few studies have been carried out suggesting that female sex hormones relax gastrointestinal smooth muscles, while androgens may induce both contraction and relaxation. In addition, no gastrointestinal contractility study with corticosteroids is available. Glucocorticoids are used in the gastrointestinal tract in Crohn's disease accompanied with motility problems [87]. The gastrointestinal application of steroids is therefore not new in clinical practice, and their motility-enhancing actions might be improved by choosing compounds with better rapid action. Intensive research is necessary to clarify the non-genomic actions of steroid hormones on contractility in the gastrointestinal system, focusing on different tracts from the stomach to the large intestine. Androgens have a unique rapid gallbladder-relaxing effect that may also be an asset in the clinical field.

The rapid actions of sex steroids may be promising in urinary bladder disorders. Estrogens and androgens elicit a relaxing effect that might be beneficial in acute increases in bladder tone. Interestingly, progesterone increases tone, which might be useful in bladder atony. The development of analogues of these hormones with strong non-genomic action may open a new direction in urinary bladder therapy. These effects might be clinically helpful in the diagnosis and/or treatment of conditions associated with abnormal bladder tone, such as urinary incontinence, bladder atony, and others. However, there is no information about the rapid action of corticosteroids on urinary bladder contractions.

The application of the non-genomic smooth muscle relaxant effect of bile acids seems theoretical at present, but further developments in this specific area may have therapeutic potential in the future for gastrointestinal, gallbladder, and urinary tract smooth muscles.

Although the therapeutic application of the rapid effects of steroid hormones and their analogues for smooth muscle contractility disorders seems remote, the actions and mechanism discovered so far are promising. Further research is needed to expand our knowledge in this field by using existing experience. One of the greatest challenges is to separate genomic and non-genomic effects, but model molecules are available to start this line of research.

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Declaration of competing interest

The authors declare they have no conflicts of interest.

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Figures legends

Figure 1: Genomic and non-genomic pathways of sex steroids in smooth muscles. The genomic pathways are initiated mainly by the binding of sex steroids with intracellular nuclear receptors, followed by the gene transcription process, while the rapid non-genomic pathways are initiated by the binding of sex steroids with the 7TM receptors or ion channels. The binding of the specific sex steroid with 7TM receptors activates or inhibits the AC enzyme and the subsequent cAMP/PKA pathway or PLC enzyme and the subsequent IP3, DAG/PKC pathways, which consequently change the ion channel activity or the homeostasis of intracellular ions (mainly calcium ions), which in turn leads to a rapid change in smooth muscle tone. The binding with membrane receptors affects eNOS enzyme activity and subsequently changes smooth muscle tone. These rapid non-genomic pathways sometimes interfere with each other and lead to some genomic responses which need more time to appear. Black lines: Stimulation, Red lines: Inhibition.

AC: Adenylyl cyclase, Akt: Protein kinase B, AMP: Adenosine monophosphate, ATP: Adenosine triphosphate, cAMP: Cyclic adenosine monophosphate, CREB: cAMP-response element binding protein, DAG: Diacyl-glycerol, eNOS: Endothelial nitric oxide synthase, Epac: Exchange protein activated by cAMP, ERK: Extracellular signal-regulated kinases, IP3: Inositol triphosphate, IP3R: Inositol triphosphate receptor, MEK: Mitogen-activated protein kinase kinase (MAP2K or MAPKK), mR: Membrane receptors, PDE: phosphodiesterase, PI3K: Phosphatidylinositol 3-kinase, PKC: protein kinase C, PL: Phospholipids, PLC; Phospholipase-C, Raf1; proto-oncogene serine/threonine-protein kinase 1 (c-Raf), Rap1: Repressor/activator protein 1, R-RAS: Small GTPase of the Ras family, RyR: Ryanodine receptor, SERCA: Sarcoplasmic/endoplasmic reticulum Ca2+-ATPase, SR: Sex steroid receptor, 7TM: Seven transmembrane domain

Figure 2: Genomic and non-genomic pathways of corticosteroids in smooth muscles. The genomic pathways are initiated mainly by the binding of corticosteroids with intracellular nuclear receptors, followed by the gene transcription process, while the rapid non-genomic pathways are initiated by the binding of corticosteroids with the 7TM receptors or membrane receptors. The binding of the specific corticosteroid with 7TM receptors activates the AC enzyme and the subsequent cAMP/PKA pathway or PLC enzyme and the subsequent IP3, DAG/PKC pathways, which consequently change the ion channel activity or the homeostasis of intracellular ions (mainly calcium ions), which in turn leads to a rapid change in smooth muscle tone. The binding with membrane receptors also affects PLC enzyme activity and subsequently changes smooth muscle tone via IP3 and Ca2+ levels in addition to the DAG/PKC pathway. These rapid non-genomic pathways sometimes interfere with each other and lead to some genomic responses which need more time to appear. Black lines: Stimulation, Red lines: Inhibition.

AC: Adenylyl cyclase, AMP: adenosine monophosphate, ATP: adenosine triphosphate, cAMP; Cyclic adenosine monophosphate, CREB: cAMP-response element binding protein, DAG: Diacyl-glycerol, Epac; Exchange protein activated by cAMP, ERK: Extracellular signal-regulated kinases, GR: Glucocorticoid receptor, IP3: Inositol triphosphate, IP3R: Inositol triphosphate receptor, MEK: Mitogen-activated protein kinase kinase (MAP2K or MAPKK), mR; Membrane receptors, PDE: phosphodiesterase, PKC: protein kinase C, PL: Phospholipids, PLC: Phospholipase-C, Raf1; proto-oncogene serine/threonine-protein kinase 1 (c-Raf), Rap1: Repressor/activator protein 1, R-

RAS: Small GTPase of the Ras family, RyR; Ryanodine receptor, SERCA; Sarcoplasmic/endoplasmic reticulum Ca2+-ATPase, 7TM: Seven transmembrane domain.

Table 1: Identified membrane receptors for steroid hormones. Bolded receptors are involved in the contractility response of non-vascular smooth muscles.

Steroid hormones	Receptor name	References
androgens	**ZIP9**, **GPRC6A**, OXER1, TRPM8	[9–12]
estrogens	**GPER1 (GPR30)**, **mERα**, mERβ, ER-X, Gq-mER	[10,13],
progestogens	**mPRα (PAQR7), mPRβ (PAQR8)**, mPRδ (PAQR6), mPRγ (PAQR5), mPRε (PAQR9), PGRMC1	[10,14]
glucocorticoids	**mGR**, CAV1	[15,16]
mineralocorticoids	-	[12]
bile acids	**TGR5**	[17]

ZIP9: ZRT-and Irt-like Protein 9; GPRC6A: G-protein-coupled receptor family C group 6 member A; OXER1: oxo-eicosanoid receptor 1; GPER1: G-protein-coupled estrogen receptor 1; GPR30: G-protein-coupled receptor 30; mER: membrane-associated estrogen receptor, ER-X: estrogen receptor X; , Gq-mER: Gq-coupled membrane estrogen receptor; mPR: membrane-associated progesterone receptor; PAQR: progestin and adipoQ receptor; PGRMC1: progesterone receptor membrane component 1; mGR: membrane-associated glucocorticoid receptor; CAV1: caveolin-1 receptor; TGR5: Takeda G protein-coupled receptor 5 receptor

Table 2: Non-genomic effects of female sex hormones on bronchial muscle

Female sex hormones	Tissue	Stimulus	Effect	Mechanism	References
17β-Estradiol	HTM	Hi	Relaxation	cAMP/PKA activation, $[Ca^{+2}]_i$ reduction	[39]
	GPTM	KCl	Relaxation	L-VDCC blockade	[33]
Progesterone	GPTM	Hi, CCh	Relaxation	L-VDCC blockade	[40]

cAMP: Cyclic adenosine-mono-phosphate, CCh: Carbachol, $[Ca^{+2}]_i$: intracellular calcium ion, GPTM: Guinea pig tracheal muscle, HASM: Human tracheal muscle, Hi: histamine, L-VDCC: L-type voltage-dependent calcium channel, PKA: Protein kinase A

Table 3: Non-genomic effects of androgens on bronchial muscle

Androgens	Tissue	Stimulus	Effect	Mechanism	References
Testosterone	RBTM	Ach, CCh	Relaxation	NO increase	[41,42]
	GPTM	CCh, KCl	Relaxation	L-VDCC, SOCC, ROCC blockade	[33,41,43,44]
	GPTM	CCh	Relaxation	IP_3Rs inhibition	[44]
5α-and 5β-DHT	RBTM	ACh, CCh	Relaxation	NO increase	[41,42]
	GPTM	CCh, KCl	Relaxation	L-VDCC, SOCC, ROCC blockade	[43,44]
	BTM	CCh	Relaxation	L-VDCC blockade	[43]

DHEA	GPTM	KCl	Relaxation	L-VDCC blockade	[33]

Ach: Acetylcholine, BTM: Bovine tracheal muscle, DHEA: Dehydroepiandrosterone, CCh: Carbachol, GPTM: Guinea pig tracheal muscle, IP_3R: Inositol triphosphate receptor, L-VOCC: L-type voltage-dependent calcium channel, NO: Nitric oxide, ROCC: Receptor-operated calcium channel, RBTM: Rabbit tracheal muscle, SOCC: Store-operated calcium channel

Table 4: Non-genomic effects of female sex hormones on uterine muscles

Female sex hormones	**Tissue**	**Stimulus**	**Effect**	**Mechanism**	**References**
17β-Estradiol	RBU	oxytocin	Contraction	Ca^{2+} increase	[45]
	RU, RPU	KCl	Relaxation	GPR30 and cAMP increase, Ca^{2+} decrease	[46,47]
Progesterone	RU, RPU	KCl	Relaxation	Ca^{2+} decrease	[47]
	RU, RPU	KCl	No effect	-	[46]
	HU, HPU	Spontaneous, KCl	Relaxation	Ca^{2+} decrease	[48]

cAMP: Cyclic adenosine monophosphate, GPR30: G-protein-coupled receptor 30, HU: Human uterus, HPU: Human pregnant uterus, RBU: Rabbit uterus, RU: Rat uterus, RPU: Rat pregnant uterus

Table 5: Non-genomic effects of androgens on uterine muscle

Androgens	Tissue	Stimulus	Effect	Mechanism	References
Testosterone	HU, HPU	Spontaneous, KCl	Relaxation	$[Ca^{+2}]_i$ and Ca^{2+} entry decrease	[41,42,48]
	RU, RPU	KCl	Relaxation	Ca^{2+} entry decrease, GPER30 and cAMP activation	[46,47]
5α-and 5β-DHT	HPU	Spontaneous	Relaxation	L-VDOC blockade	[48]
DHEA	HPU	Spontaneous, KCl	Relaxation	L-VDOC blockade	[48]

$[Ca^{+2}]_i$: intracellular calcium ion, cAMP: Cyclic adenosine monophosphate, DHEA: Dehydroepiandrosterone, GPR30: G-protein-coupled receptor 30, HU: Human uterus, HPU: Human pregnant uterus, L-VOCC: L-type voltage-dependent calcium channel, RU: Rat uterus, RPU: Rat pregnant uterus

Table 6: Non-genomic effects of female sex hormones on gastrointestinal smooth muscles

Female sex hormones	Tissue	Stimulus	Effect	Mechanism	References
17β-Estradiol	RS	Spontaneous	Relaxation	NO/cGMP activation	[49]
	GPGB	CCK, KCl	Relaxation	Ca^{2+} entry inhibition	[50]
Progesterone	DC	KCl	Relaxation	cAMP, Ca^{2+} entry inhibition	[51]
	GPGB	CCK, KCl	Relaxation	Ca^{2+} entry inhibition	[50]

DC: Dog colon, CCK: cholecystokinin, cGMP: Cyclic guanosine monophosphate, GPGB: Guinea pig gallbladder, NO: nitric oxide, RS: Rat stomach

Table 7: Non-genomic effects of androgens on gastrointestinal smooth muscles

Androgens	**Tissue**	**Stimulus**	**Effect**	**Mechanism**	**References**
Testosterone	MI, MC	Spontaneous, $CaCl_2$, CCK	Contraction	Rho/ROCK activation	[52,53]
	GPGB	CCK	Relaxation	Ca^{2+} entry inhibition, block of IP_3-dependent $[Ca^{2+}]_i$ increase	[54]
5α-and 5β-DHT	MI	$CaCl_2$, CCh	Contraction	Rho/ROCK activation	[52]
	GPGB	CCK	Relaxation	Ca^{2+} entry inhibition, block of IP_3-dependent $[Ca^{2+}]_i$ increase	[54]

$[Ca^{+2}]_i$: intracellular calcium ion, CCh: Carbachol, CCK: Cholecystokinin, GPGB: Guinea pig gallbladder, IP_3: Inositol triphosphate, MC: Mouse colon, MI: Mouse ileum, Rho/ROCK: Rho/Rho-associated coiled-coil containing protein kinase

Table 8: Non-genomic effects of sex hormones on urinary tract smooth muscles

Sex hormones	**Tissue**	**Stimulus**	**Effect**	**Mechanism**	**References**

17β-Estradiol	RBUB	Spontaneous	Relaxation	Ca^{2+} entry inhibition	[56]
Progesterone	RBUB	Spontaneous	Contraction	Ca^{2+} entry activation	[56]
Testosterone	RUB	Spontaneous, CCh, Ap5A	Relaxation	Inhibition of neuromuscular transmission	[57]
	GPUBC	Spontaneous	Hyperpolarization	BK channel activation	[58]

Ap5A: Diadenosine pentaphosphate (P2X receptor agonist), BK channels: Large conductance voltage- and Ca^{2+}-activated K^+ channels, CCh: Carbachol, GPUBC: Guinea pig urinary bladder cells, RBUB: Rabbit urinary bladder, RUB: Rat urinary bladder

Table 9: Non-genomic effects of glucocorticoids on bronchial smooth muscle

Glucocorticoids	**Tissue**	**Stimulus**	**Effect**	**Mechanism**	**References**
Cortisol, dexamethasone	GPTM	Hi	Relaxation	PKC activation, non-genomic (not defined)	[63,64]
Budesonide	GPTM	Ovalbumin	Relaxation	Non-genomic (not defined)	[65]
Beclomethasone	HOL	LTC4	Relaxation	Non-genomic (not defined)	[66]
Prednisone, fluticasone, budesonide	HASMC	Spontaneous	Relaxation	$G_{\alpha s}$ stimulation, cAMP increase	[32]

cAMP: Cyclic adenosine monophosphate, Gsα: G-protein α subunit, GPTM: Guinea pig tracheal muscle HASMC: Human airways smooth muscle cells, HOL: Horse lung, LTC4: Leukotriene C4, PKC: Protein Kinase C

Table 10: Non-genomic effects of corticosteroids on uterine smooth muscle

Corticosteroids	Tissue	Stimulus	Effect	Mechanism	References
Glucocorticoids	RU	KCl	Relaxation	Ca^{2+} influx decrease	[47]
	RU, RPU	KCl	Relaxation	cAMP increase	[79]
	RU	Spontaneous	Relaxation (mM cc.) Contraction (nM cc.)	Non-genomic (not defined)	[45]
Mineralocorticoids	RU, RPU	KCl	Relaxation	cAMP increase	[79]

cAMP: Cyclic adenosine monophosphate, cc.: concentration, RU: Rat uterus, RPU: Rat pregnant uterus

Table 11: Non-genomic effects of corticosteroids on gastrointestinal smooth muscle

Corticosteroids	Tissue	Stimulus	Effect	Mechanism	References

Glucocorticoids	HCEC	Spontaneous	Not investigated	-	[80,81]
Mineralocorticoids	RC	CCh, EFS	No effect	-	[82]
	HCEC	Spontaneous	Not investigated	PKC activation Ca^{2+} entry increase	[80,81]

CCh: Carbachol, EFS: Electric field stimulation, HCEC: Human colonic epithelial cells, RC: Rat colon, PKC: Protein kinase C