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Frontiers in Neuroendocrinology 29 (2008) 182–198
Frontiers in Neuroendocrinology
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Review

Rapid actions of androgens

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Available online 5 October 2007

Abstract

The biological activity of androgens is thought to occur predominantly through binding to intracellular androgen-receptors, a member of the nuclear receptor family, that interact with specific nucleotide sequences to alter gene expression. This genomic-androgen effect typically takes at least more than half an hour. In contrast, the rapid or non-genomic actions of androgens are manifested within in seconds to few minutes. This rapid effect of androgens are manifold, ranging from activation of G-protein coupled membrane androgen-receptors or sex hormone-binding globulin receptors, stimulation of different protein kinases, to direct modulation of voltage- and ligand gated ion-channels and transporters. The physiological relevance of these non-genomic androgen actions has not yet been determined in detail. However, it may contribute to modulate several second messenger systems or transcription factors, which suggests a cross-talk between the fast non-genomic and the slow genomic pathway of androgens. This review will focus on the rapid effects of androgens on cell surface and cytoplasmic level.

Keywords: Androgens; Testosterone; Non-genomic effects of androgens; Acute actions of androgens

1. Introduction

Androgens are important male sex steroid hormones that exert many physiological roles leading to the male characteristics and other phenotypes. The major circulating androgen is testosterone in human tissue. Two weak androgens, dehydroepiandrosterone and androstenedione are mostly synthesized in adrenal glands (in small amounts also in the brain). They exceed about 5–10% activity of testosterone [124]. Androstenedione is converted into testosterone mainly in testis Leydig cells and peripheral tissue, or aromatized into estradiol. Testosterone is metabolized by 5α-reductase in the potent androgen 5α-dihydrotestosterone and like androstenedione in estradiol by P450-aromatase (also called estrogen synthase) (Fig. 1).

In the classical model of steroid action, steroid hormones such as testosterone and its metabolite 5α-dihydroxytestosterone diffuse through the plasma membrane and bind to intracellular androgen receptors (ARs).[1] The ARs are members of the nuclear receptor superfamily,

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[1] *Abbreviations used:* 7TMR, seven-transmembrane receptor; 8-bromo-cGMP, 8-bromo-guanosine 3,5-cyclic monophosphate; ABC, ATP-binding cassette; ABP, androgen-binding protein; AR, androgen receptor; CAD, coronary artery disease; cAMP, cyclic adenosine-3,5-monophosphate; CREB, cAMP response element-binding protein; DAG, diacylglycerol; DHEA, dehyroepiandrosterone; ER, endoplasmic reticulum; Erk, extracellular signal-regulated protein kinase; FAK, focal adhesion kinase; GDP, guanosine diphosphate; G-protein, guanine nucleotide-binding protein; GPCR, G-protein coupled receptor; GTP, guanosine triphosphate; GRD, GTPase-responsive domain; IP_3, inositol-1,4,5-triphosphate; JNK, c-Jun NH_2-terminal kinase; K_{ATP}, ATP-dependent potassium channel; Kir, inward rectifier potassium channel; LNCaP, lymph node carcinoma derived from human prostate; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein/extracellular signal-regulated kinase; NIPP-1, nuclear inhibitor of protein phosphatase-1; PI-3K, phosphatidylinositol-3 kinase; PIP_2, phosphatidylinositol-4,5-biphosphate; PIP_3, phosphatidylinositol-3,4,5-triphosphate; PKA/B/C, protein kinase A/B/C; PLA/C, phospholipase A/C; PP-1N, protein phosphatase-1N; PSA, prostate-specific antigen; PTX, pertussis toxin; RyR, ryanodine receptor; SAPK, stress-activated protein kinase; SHBG, sex hormone binding globulin; SHBG-R, SHBG-receptor; SOS, son of sevenless; SR, sarcoplasmic reticulum; Src, steroid receptor coactivator.

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doi:10.1016/j.yfrne.2007.08.004

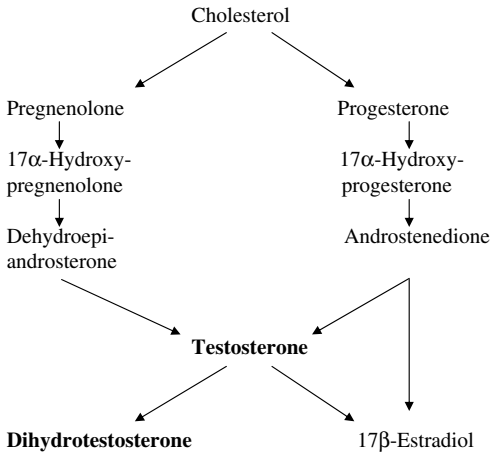

Fig. 1. Biosynthetic pathways of androgens. The androgen synthesis starts from cholesterol as the precursor. Two androgens, dehydroepiandrosterone and androstenedione are mainly produced in adrenal tissue. Androstenedione is converted into testosterone or estradiol mainly in testis and peripheral tissues. Testosterone is further being changed to the more potent 5α-dihydrotestosterone by 5α-reductase, or converted into estradiol by aromatase.

which function as ligand-inducible transcription factors. The binding of androgens to ARs induces receptor dimerization, facilitating the ability of ARs to bind to specific DNA sequences, called hormone response element, and recruit coregulators to promote the expression of target genes [78,79,167]. In addition to this slow genomic mode of action by steroids, increasing evidence suggests that androgens, as well progesterone and estrogens, can exert rapid non-genomic effects [32,50,56,104,135,158,169,204].

The nature of the steroid-induced signal (genomic versus non-genomic) depend on the type of target cell, the receptor location within cells, as well as the ligand itself [120]. The rapid action by steroid hormones received attention in 1967, when Szego and Davis reported that physiologic doses of 17β-estradiol given intravenously increased the uterine cyclic adenosine-3,5-monophosphate (cAMP) concentration in ovariectomized mice within 15 s [191]. Koenig et al. reported that physiological concentrations of testosterone (1–10 nM) induce a rapid (<1 min) stimulation of endocytosis, amino acid and hexose transport in mouse kidney cortex [104]. Testosterone induced a fast (<5 s), short-lived rise in ornithine decarboxylase activity and in polyamine concentration, as well as an acute (30–60 s) stimulation of Ca^{2+} fluxes and Ca^{2+}-dependent membrane transport [103]. These rapid effects of androgens are characterized by (i) their short latency (seconds or minutes), and (ii) the speed of the response, which suggest no direct involvement of nuclear receptors. The non-genomic pathway of androgens typically involves rapid changes on cell membrane or cytoplasmic level. Rapid actions on membranes may be a direct influence of ion-channels and transporters (e.g. Ca^{2+}- or Na^{+}–K^{+}-ATPase) [50,51,64,135,221] or an indirect modulation by induction of conventional second messenger signal transduction cascades, including increases in free intracellular Ca^{2+}, activation of protein kinases A/C (PKA/PKC) or mitogen-activated protein kinase (MAPK) [106,160]. The initiation of these second messenger cascades modulate indirectly ion-channels but also mediate changes on transcription level [72,151].

The non-genomic action of androgens has been implicated in a number of cellular effects, including intracellular Ca^{2+}-hemostasis, gap junction communication, aortic relaxation, neuronal plasticity or neurite outgrowth [36,53,54,107,114,153,212]. However, a lot of experiments were conducted at unphysiological androgen levels (>1 μM) and only few studies were performed within a physiological range (10–25 nM). Moreover, it has to be considered that aging is associated with a progressive decline in serum testosterone levels. Total testosterone levels <7 nM confirm late-onset hypogonadism in the ageing male and indicate that benefits may be derived from testosterone long-term treatment. Recently, some studies demonstrated an acute benefit of testosterone in myocardial ischemia [92,155,161,203]. However, the physiological and clinical relevance of such acute administrations of testosterone are still unknown.

The non-genomic effect of steroids could potentially be mediated (i) by direct binding to a specific-binding site of the target molecule, in the absence of a AR, (ii) through the classical intracellular-AR, e.g., activation of Src kinase, (iii) by a distinct non-classical transmembrane-AR, i.e., transmembrane G-protein coupled receptor, or (iv) via changes in membrane fluidity. The first three mechanisms are often used to explain the underlying rapid effects of steroid hormones, whereas the last non-specific effect on membrane fluidity is often neglected. Hydrophobic steroids can interact with the polar heads of membrane phospholipids [45,209]. The accumulation of androgens in the membrane could influence cellular adhesion, cell–cell interaction, and the function of ion-channels [45,209]. Besides androgens, also estrogens, progesterone or 5α-dihydrotestosterone have been found to alter membrane fluidity in cell types without classical steroid receptors [33,45,195]. Therefore another potential non-genomic steroid mechanism is the intercalation of the lipophilic steroids into the membrane of target cells, resulting in pertubation of lipid–lipid interactions that may, in turn, alter the function of membrane proteins. Steroid hormones intercalate into the membrane bilayer, with their planar ring system in the hydrophobic core of the bilayer, lying parallel with the phospholipid molecules [108]. In one study, Whiting et al. demonstrated the effect of steroids in changing the dynamics of the membranes on integral activity using Ca^{2+}-ATPase from sarcoplasmic reticulum membranes. Cholesterol decreases the activity of Ca^{2+}-ATPase, whereas progesterone, 17β-estradiol and testosterone increases the activity [207]. Alterations in the lipid fluidity of the membranes may therefore directly influence the activity of integral proteins through a change in their conformational flexibility.

A simple scheme for rapid modulation by steroids that tried to describe the mechanism of such rapid signaling is

the "Mannheim classification" [55]. Based on how androgens can bind to specific or non-specific receptors and directly or indirectly, this scheme is divided into two major groups termed A (direct steroid action, e.g., direct effect on ion-channels) and B (indirect steroid action, e.g., G-protein coupled mechanism), which are subdivided into a (I) non-specific and a (II) specific category. The class II is further divided into group (IIa) classic steroid receptor (e.g., by intracellular-ARs) and (IIb) non-classic steroid receptor group (e.g., through SHBG-R). Because androgens acts not only on one way and these pathways are connected to each other, we preferred in our review a disposition based on target proteins and major pathways, respectively.

2. PKA/PKC-mediated rapid effect of androgens

Heterotrimeric guanine nucleotide-binding proteins (G-proteins) are signal transducers, attached to the cell membrane, that connect receptors to effectors and thus to intracellular signaling pathways [165]. G-proteins consist of three subunits, α, β and γ. Around 21 Gα, 6 Gβ, and 12 Gγ subunits exist. Receptors that couple to G-proteins communicate signals from a large number of hormones, neurotransmitters, chemokines, autocrine or paracrine factors. Binding of these molecules to these receptors activates G-proteins, including the stimulatory G-protein (G_s), the inhibitory G-protein (G_i), the G_q-protein and the G_{12}-protein. G_s- and G_i-proteins regulate mainly adenylate-cyclases that produce cAMP, G_q regulates phospholipases that control intracellular Ca^{2+} levels, and G_{12} regulates low molecular weight GTPase Rho and other effectors. When signaling, G-proteins function as dimers because the signal is communicated either by the Gα subunit or the Gβγ complex.

The actions of hormones and neurotransmitters are mediated by membrane localized ARs or by cell surface receptors with a seven-transmembrane topology, called seven-transmembrane receptors (7TMRs). The existence of transmembrane-ARs on the cell surface has been presumed by various authors based on the detection of specific androgen-binding sites [7,10,29,79]. The localization of membrane-ARs is less well characterized, although membrane-ARs has been found to be associated with caveolin, an integral membrane protein that serves as a scaffolding protein for many signaling molecules, including phosphatidylinositol-3 kinase (PI-3K), Ras, and Src [119,146]. Cheng et al. demonstrated that testosterone (100 nM) induced a rapid (5 min) and transient AR migration to the cell membrane, which support the existence of transmembrane-ARs [29]. The membrane-ARs transmit their signals via G-proteins, therefore often called G-protein coupled receptors (GPCR). After agonist binding to its receptor, the Gα subunit releases guanosine diphosphate (GDP), binds guanosine triphosphate (GTP) and dissociates from the Gβγ protein complex [181]. In most cases, Gβγ subunits cannot be dissociated under non-denaturing conditions, and regulate activity of many effectors, including phospholipases (e.g., PLC) and serine–threonine (e.g., Raf) and tyrosine kinases (e.g., c-Src) [152]. Effector activation occurs at the cell surface membrane, which results in the synthesis of second messengers (e.g., Ca^{2+}, IP_3 or cAMP) that diffuse intracellular and trigger different pathways including protein phosphorylation or gene transcription [145]. Androgens can activate such second messengers by modulating GPCR [8,98,112,114].

The majority of serum androgens (approximately 60%) are complexed with the plasma glycoprotein sex hormone-binding globulin (SHBG) and androgen-binding protein (ABP), and a minority is bound to albumin [20,133,177]. ABP and SHBG are encoded by the same gene, and both proteins have the identical amino acid sequence within a species, but are likely to originate from alternative processing and glycosylation mechanisms controlled by tissue-specific regulatory elements [60,159]. In the following text, we will use the term SHBG. SHBG is a multifunctional protein that acts in humans to regulate the response to steroids at several cross-points and the bioavailability of free steroids to hormone-responsive target cells. The SHBG is located on membranes of sex steroid responsive cells like in epididymis, testis, prostate, skeletal muscle, liver cells, neurons and cardiomyocytes [6,22,63,81,171]. Therefore, many groups suggest that the non-genomic effect is mediated by such G_s-protein coupled SHBG-receptor (SHBG-R) through the modulation of adenylate-cyclase with cAMP synthesis and activation of PKA [70,142,163,164]. The SHBG/SHBG-R system works as an additional control mechanism which inhibits or amplifies the effects of androgens and estrogens in target cells. For androgens to induce cAMP through the SHBG/SHBG-R system, SHBG must first bind to the SHBG-receptor and then bind to the androgen molecule. SHBG already bound to steroid are not able to interact with the SHBG-R [163,164]. The SHBG/SHBG-R system functions as part of a novel steroid-signaling system that is independent of the classical intracellular steroid receptors [93]. Androgens and estrogen are able to rapidly (5–15 min) induce cAMP by this pathway [60,61,154,162]. In the prostate, it has been suggested that the estradiol-activated SHBG/SHBG-R complex cross-talks with the AR, and is able to activate ARs even in the absence of androgens [60]. In serum free media, the addition of SHBG and the presence of cellular SHBG-R are necessary for steroid induction of cAMP [60]. A SHBG-cAMP-mediated activation of PKA is the result in different target cells, e.g., prostate, breast, placenta, liver, or epididymis cells [4,61,62,71,143]. The PKA pathway is particulary interesting because of its possible ability to modulate ARs and stimulate the expression of the AR-regulated genes, such as prostate-specific antigen (PSA) [67,102,170]. The induction of PSA expression occurs in response to 5α-dihydrotestosterone, which could be mimicked by treatment with both SHBG and estrogen, while SHBG or estrogen alone had no effect [143]. Estrogen-SHBG stimulation of PSA secretion was not inhibited by anti-estrogens but was abolished by anti-androgens [143].

Like other steroid hormone receptors, the AR is a phosphoprotein [90]. Phosphorylation of the AR was first described by the group of van Laar [199,200]. A 1.8-fold increase in phosphorylation of the AR was observed after 30 min under androgen treatment (R1881, 10 nM) of lymph node carcinoma cells derived from human prostate (LNCaP) [199]. It is known, that the AR is synthesized as a non-phosphorylated protein and migrates as a 110 kDa protein. Within 15 min after synthesis, the non-phosphorylated AR becomes phosphorylated and becomes a 110–112 kDa doublet [109]. Amino acids residues which are known to be involved in AR phosphorylation are serine residues located at positions 16, 80, 81, 93, 94, 256, 308, 424, 641, and 650 [28,67,90,217,219]. Blok et al. demonstrated that PKA activation dephosphorylate ARs within 10–30 min [15,16]. The rapid mechanism suggests that AR dephosphorylation is an active process which involves activation of phosphatases rather than inhibition of protein kinases. Indeed, the activity of some phosphatases is known to be regulated by PKA stimulation. For example, the nuclear protein phosphatase-1 (PP-1N) is activated by PKA-induced phosphorylation of nuclear inhibitor of protein phosphatase-1 (NIPP-1) [12,197,198]. The dephosphorylation of the AR may involve this phosphatase. A recent study showed that mutations on the phosphorylation sites did not have an effect on AR transcriptional activity [67]. On the one hand, phosphorylation sites may regulate nuclear transport, and not transcription [14]. On the other hand, PKA enhancement of androgen-mediated transcription is not due to increased AR expression or stabilization [102]. Other targets of the SHBG/androgen-induced PKA activation are ion-channels and/or transporters.

3. MAPK-mediated rapid effect of androgens

Another important G-protein-mediated route of fast hormone-response is the MAPK-pathway, which is one of the most relevant aspect in apoptosis signaling. For example, the cytokine TNF-α induced apoptosis in a dose-dependent manner in LNCaP cells. MAPK inhibitors increased the apoptotic rate in LNCaP cells following p38 inhibition [160]. MAPK stimulation by androgens has been linked to protection from apoptosis in several cell types [106]. These protective or damage-promoting effects by androgens on the MAPK-signaling pathways are depending on whether a membrane-AR or intracellular-AR is activated [66].

The three members of the MAPK-superfamily in mammalian cells are (i) the c-Jun NH_2-terminal kinases, or stress-activated protein kinase (JNK/SAPK), (ii) the stress-activated protein kinase 2, or reactivating protein kinase (p38), and (iii) the extracellular signal-regulated protein kinases (Erk-1/2, p44/p42). These protein kinases are recognized as playing a key role in cell growth, differentiation and function at both the transcriptional and post-transcriptional level, by phosphorylating a range of proteins including nuclear transcription factors, cytoskeletal proteins, other protein kinases, protein phosphatases, and receptors for hormones and growth factors [38]. Albeit with multiple exceptions, JNK/SAPK and p38 kinase are generally associated with apoptosis induction, while Erk-1/2 are generally associated to mitogenesis, and therefore inversely related to apoptosis [39].

Androgens are able to modulate Erk-1/2 via AR and GPCR within seconds [151], whereas neither JNK/SAPK nor p38 kinase activity were altered by 5α-dihydrotestosterone [215], the synthetic androgen R1881 [218] or dehyroepiandrosterone [208], suggesting an androgen-specific MAPK → Erk-1/2 pathway. G-proteins or small GTPases, like Ras or Rap1, are able to activate other effectors such as protein kinase cascades that include members of the MAPK-family. Rap-1 is a small GTPase most closely related to Ras. MAPK-pathways are as well known to regulate rapid and slow effects of several steroid receptors. Once the plasmalemmal Ras is activated by inducing the exchange of GDP with GTP, it recruits Raf-1 or named c-Raf, a serine–threonine kinase. Activated Ras act as an adapter molecule that binds to Raf-1 kinases with high affinity. Raf-1 is activated when it associates with membranes. Once active, Raf-1 phosphorylates and activates the MAPK-Erk-kinase (MEK), a dual specificity tyrosine–threonine kinase, which in turn phosphorylates and activates the extracellular signal-related kinases Erk-1 and Erk-2. The Erk-proteins are serine–threonine kinases. After full activation of these enzymes Erk translocate from the cytoplasma into the nucleus and phosphorylate numerous cytoplasmic and nuclear targets, including kinases, phosphatases, transcription factors (e.g., Elk-1) and cytoskeletal proteins [213,215]. Although Rap-1, like Ras, can bind to Raf-1, the ability of Rap-1 to substitute for Ras in activating the MAPK pathway has been controversial [18]. Some studies indicated that Rap-1 can indeed activate Erk-1/2 in a Ras-independent fashion [138,185]. Other recent observations include the potential role of tyrosine kinase c-Src in the activation of Rap through cAMP-dependent PKA.

Androgens and estrogens can rapidly enhance the kinase activity of estrogen and androgen receptors, which then activates MAPK via the Ras pathway [23,52,151,180,188,215]. Several steroid hormones have been shown to stimulate Erk-1/2 phosphorylation [23,88,151]. 5α-dihydrotestosterone led to a rapid and reversible activation of Erk-1/2 via AR in prostate cancer cells [151], whereas the effect of dehyroepiandrosterone (DHEA) in vascular smooth muscle cells was not mediated by either AR or estrogen-receptor but possibly via a DHEA-specific receptor [208]. The skeletal muscle Erk-1/2 can be activated by exercise, stress stimuli, growth factors or hormones [139,144]. Stimulation of such skeletal muscle cells with testosterone (10 nM–1 μM) and 19-nor-testosterone (100 nM) elevated the phosphorylation comparable rate of Erk-1/2 within few minutes (peak after 5 min). The androgen-induced phosphorylation of Erk-1/2 at 5 min was 2.0- to 2.5-fold increased in comparison to the basal values [52]. Furthermore, testosterone conjugated

to bovine serum albumin, which does not cross the plasma membrane and binds to membrane-AR, led to an increase of Erk-1/2 phosphorylation comparable to that of free testosterone [52]. The fast and transient phosphorlyation was not inhibited by the AR-antagonist cyproterone (1 μM) but blocked by inhibiting the intracellular inositol-1,4,5-triphosphate (IP_3)-mediated Ca^{2+}-release or with the MEK-inhibitor PD-98059 (10 μM). In addition, under pertussis toxin (PTX), which prevents the G-protein heterotrimers from interacting with G-protein coupled receptors, Erk-1/2 phosphorylation was blocked, suggesting an AR-independent but Ca^{2+}-dependent phosphorylation of Erk-1/2 [52]. Further, the effect of testosterone in dominant negatives of MEK and Ras proteins was inhibited, indicating a PTX-sensitive G-protein coupled $\rightarrow PLC \rightarrow IP_3 \rightarrow [Ca^{2+}]_i \rightarrow MEK \rightarrow Erk$ as well $Ras \rightarrow Raf \rightarrow MEK \rightarrow Erk$ signaling [52]. In this Ras-MEK pathway the extracellular signal is transmitted from the cell surface to the nucleus, and a transcriptional program is finally activated (Fig. 2) [139].

4. PI-3K-mediated rapid effect of androgens

Androgens can also activate the phosphatidylinositol-3 kinase/Akt (PI-3K/Akt) pathway rapidly in a ligand-binding independent manner [95,106,120,189,205]. Activation of PI-3K by Src-family kinases has been documented [116]. PI-3K is a kinase, which is composed of a p85 regulatory and a p110 catalytic subunit. The p85-subunit contains a GTPase-responsive domain (GRD) and an inhibitory domain, which together form a molecular switch that regulates PI-3K [26]. The association of small GTPase proteins with the GRD domain of p85 is also important for the activation of PI-3K [189]. Ras has been shown to bind and activate PI-3K in a GTP-dependent manner. Activated PI-3K catalyzes the phosphorylation of phosphatidylinositol-4,5-biphosphate (PIP_2) to yield phosphatidylinositol-3,4,5-triphosphate (PIP_3) in response to many growth factors and cytokines. PI-3K and their lipid products act on pathways that control cell proliferation, cell survival

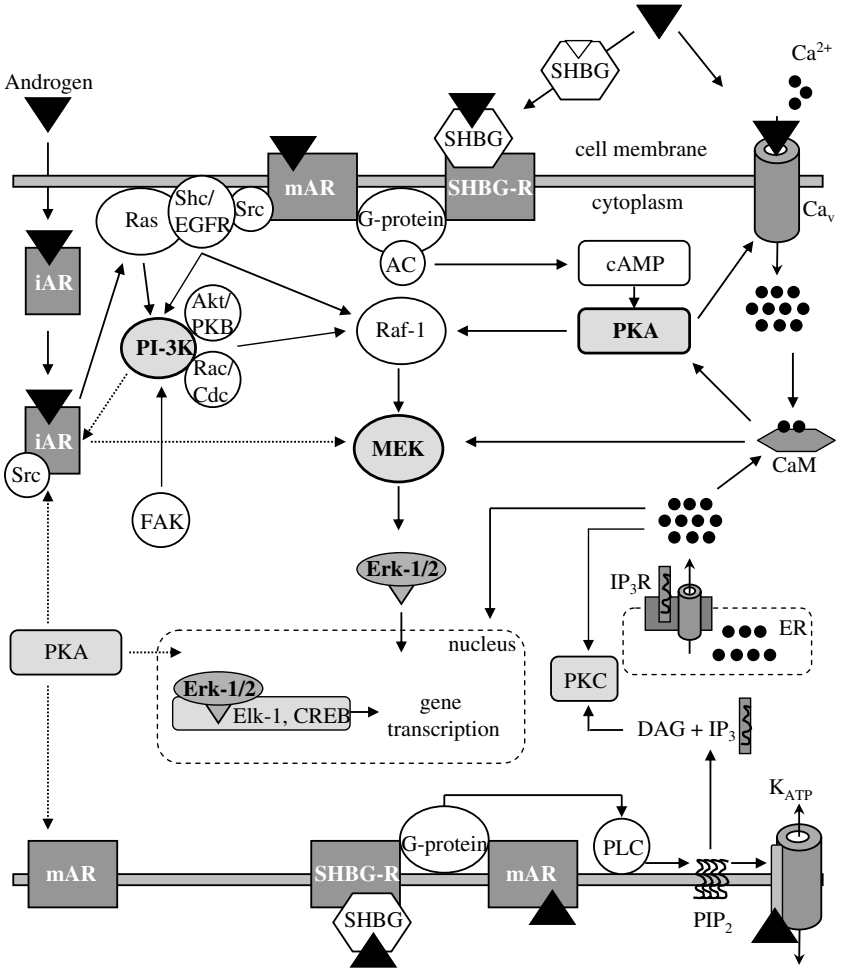

Fig. 2. Non-genomic, rapid actions of androgens. *Abbreviations:* CaM, calmodulin; cAMP, cyclic adenosine-3,5-monophosphate; Ca_v, voltage-gated Ca^{2+}-channel; CREB, cAMP response element-binding protein; DAG, diacylglycerol; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; Erk, extracellular signal-regulated protein kinase; FAK, focal adhesion kinase; G-protein, guanine nucleotide-binding protein; iAR, intracellular-androgen receptror; IP_3, inositol-1,4,5-triphosphate; IP_3R, IP_3 receptor; K_{ATP}, ATP-dependent K^+-channel; mAR, membrane-androgen receptor; MEK, mitogen-activated protein/extracellular signal-regulated kinase; PI-3K, phosphatidylinositol-3-kinase; PIP_2, phosphatidylinositol-4,5-biphosphate; PKA/B/C, protein kinase A/B/C; PLC, phospholipase C; SHBG, sex hormone binding globulin; SHBG-R, SHBG-receptor; Src, steroid receptor coactivator.

and metabolic changes, often through two different protein kinases (i) protein kinase B (PKB) and (ii) p70 ribosomal protein S6 kinases ($p70^{S6K}$). PIP_3 directly stimulates PKB, also named Akt, causing its translocation to the membrane and enabling other kinases to phosphorylate and activate PKB. A double phosphorylation of Akt at Thr-308 and Ser-473 is necessary for full Akt-kinase activation [19]. 5α-dihydrotestosterone (10 nM) and testosterone induced a rapid phosphorylation of AR, which did not occur in response to AR-antagonist and was inhibited by the PI-3K inhibitor LY294002, suggesting a site-specific AR-phosphorylation by these intracellular kinases [192]. Some studies demonstrated clearly, that 5α-dihydrotestosterone, 17β-estradiol, 3α-diol, and testosterone induced a rapid and transient phosphorylation of Akt [1,65,95]. Androgens are also involved in the rapid modulation by the S6 kinase pathway [211].

Rac and Cdc42, which are members of the Rho-family and are involved in the organization of the actin cytoskeleton and the JNK pathway, are regulated as well by PI-3K. Rac and Cdc42 are involved in Ras transformation. Testosterone (100 nM) treatment resulted in rapid phosphorylation of focal adhesion kinase (FAK), the association of FAK with the PI-3K, and the subsequent activation of the latter as well as the activation of the small GTPases Cdc42/Rac in LNCaP cells [147]. FAK is a non-receptor tyrosine kinase that is localized in focal adhesion. The FAK → PI-3K → Cdc42/Rac1 (small GTPases) pathway is therefore responsible for the actin cytoskeleton rearrangement (Fig. 2).

5. Rapid androgen effect on transcription factors

The non-genomic, rapid stimulation of different pathways by androgen exert a biological effect through modulation of the transcriptional activity of ARs or other transcription factors. Ligand-bound ARs associate with a numerous proteins at the cell membrane, e.g., Src, caveolin-1, PI-3K, and epidermal growth factor receptor (EGFR), and activate numerous cell signaling pathways [17,24,119,137,179]. For example, stimulation of NIH3T3-fibroblasts by the synthetic androgen R1881 activates the steroid receptor coactivator (Src) kinase pathway and promotes the S-phase entry or cytoskeleton changes in these cells [24]. Recent studies have confirmed this non-genomic action of androgen depending on the ability of the AR to interact with Src. The AR-Src-complex led to Erk-2 activation by the MAPK-pathway [79,106]. The Src-MAPK is one of the major route in the signal transduction [29,106,137,194]. The non-receptor tyrosine kinase c-Src is normally targeted to the inner cell surface of the plasma membrane by myristylation and palmitoylation of its N-terminus. Cheng et al. found that a population of ARs is localized at the plasma membrane and that also these membrane-ARs associates with Src kinase after testosterone stimulation [29]. Testosterone (10–250 nM) induced a rapid activation of Src within minutes in primary rat Sertoli cells, which correlated with the increased AR-Src interactions that occur within 5 min after testosterone stimulation [29]. The Src kinase activity is autoinhibited by the interaction between the tryosine kinase domain and the Src-homology 2 (SH2) and Src-homology 3 (SH3) domains. Disruption of these intramolecular interactions by proteins binding to the SH2 or SH3 domains, or through dephosphorylation of tyrosine residues of the SH2 domain, results in activation of the Src kinase. The androgen–AR complex interacts with the SH3 domain of Src kinase and led to a rapid activation of the c-Src → Raf-1 → MEK → Erk-2 pathway [24,25,106,137]. The anti-apoptotic effect of 5α-dihydrotestosterone on transfected HeLa cells is reversed by a Src mutant lacking the SH3 domain [106], suggesting that the AR-Src interaction is necessary for the inhibition of adoptosis. Protection involves phosphorylation of the prosurvival factor Src and induction of the anti-apoptotic protein Bcl-2 [27]. One of the targets of c-Src is the adapter protein Shc. c-Src activation of Shc is known to rapidly induce the formation of Shc/Grb2/SOS complexes, leading to the stimulation of the MAPK pathway. Further, Src is also capable of directly or indirectly activating EGFR and subsequently the MAPK-pathway by phosphorylating tyrosine residues on the cytoplasmic face of EGFR [186]. Androgen-induced c-Src activation occurs only in the cell membrane free from the interaction of EGFR with AR, whereas EGFR, estrogen receptor, and c-Src were found to form a complex upon estrogen stimulation [82].

Recent studies have reported that androgens can rapidly activate transcription factors like the cAMP response element-binding protein (CREB) transcription factor in Sertoli cells [29,59]. Unni et al. demonstrated that Src kinase is activated by androgens and that Src kinase activity is required for stimulation of the c-Src → Ras → Raf-1 → MEK → Erk- 1/2 → CREB pathway [194]. Moreover, by characterizing the signaling pathway, Cheng et al. found that testosterone (>10 nM) stimulation results in AR migration to the plasma membrane, facilitates AR-Src interactions, and induces the phosphorylation and activation of the Src kinase [29]. Testosterone activated the MAPK-pathway also via epidermal growth factor on a Src → EGFR → MEK → Erk-1/2 → CREB pathway [29]. Further, the androgen induced SHBG-cAMP-mediated activation and the elevation of intracellular Ca^{2+}, which activates PKA, PKC or MAPK, can modulate the activity of transcription factors. Nevertheless, additional studies are required to understand the mechanism of these complex pathways and their interactions among each other (Fig. 2).

6. Rapid effect of androgens on intracellular Ca^{2+}-hemostasis

Ca^{2+} functions as an ubiquitous second messenger and regulates a wide range of cellular processes, including proliferation, apoptosis, motility, and gene expression [11]. The major routes to regulate the intracellular $[Ca^{2+}]_i$ are sarcoplasmic reticulum (e.g., by IP_3-receptors) and plasma membrane integrated Ca^{2+}-channels (e.g., by L-/T-type Ca^{2+}-channels). The changes in intracellular calcium level,

which are detected by specific Ca^{2+} sensors (e.g., PKC and calmodulin), have different spatial and temporal Ca^{2+} signal patterns, and release Ca^{2+} from different subcellular compartments, which may be associated with an increase in nucleoplasmic Ca^{2+} and therefore may influence the transcriptional response in target cells [40,43,54,72,113,132,175]. For example, the rapid turn on and off of Ca^{2+} signals often produces oscillations. It has been shown in skeletal muscle cells that testosterone induces intracellular Ca^{2+} oscillations, which begin as Ca^{2+} transients initiated in the cytosol and propagate as waves of Ca^{2+} in the cytoplasam and nucleus [52–54]. These complex Ca^{2+} signals depend on an interplay between IP_3-sensitive stores and Ca^{2+} influx from extracellular. Thus, a different oscillatory pattern may indicate a distinct mechanism of Ca^{2+} release and recapture as well as a different function for Ca^{2+} as an intracellular signal [43,54,113]. However, the functional importance of testosterone-induced non-genomic Ca^{2+} signaling seems to be linked to the genomic pathway. Androgens are able to modulate the intracellular Ca^{2+} level within seconds to minutes in different cell systems, including human prostata cancer cells [183], male (but not female) rat osteoblasts [114], macrophages [7,10,72], mouse T-cells [8,9], human granulosa lutenizing cells [123], rat Sertoli cells [69,121], neuroblastoma cells [54], rabbit kidney cells [37], vascular smooth muscle cell line [75], skeletal muscle cells [52] and rat cardiomyocytes [50,135,201]. The mechanism of modulation of intracellular Ca^{2+} level by androgens can be divided in an AR-sensitive and in an AR-insensitive pathway. The AR-sensitive mechanism is based on the presence of androgen receptors, whereas the AR-insensitive mechanism is explained mainly by a G-protein coupled process.

6.1. AR-sensitive modulation of intracellular Ca^{2+}-hemostasis

Steinsapir et al. were the first to show a rapid (<1 min) effect of androgens on cytosolic free Ca^{2+} [183]. In LNCaP cells dimethylnortestosterone (1 pM–1 μM) and 5α-dihydrotestosterone modulated $[Ca^{2+}]_i$ via two mechanisms: Ca^{2+} influx from the extracellular milieu and Ca^{2+} mobilization from the endoplasmic reticulum (ER). The L-type Ca^{2+}-channel antagonist verapamil (1 μM) and the non-steroidal anti-androgen hydroxyflutamide (1 mM) suppressed the androgen-induced cytoplasmic Ca^{2+} rise, suggesting an AR-dependent modulation of voltage-gated Ca^{2+}-channels. A rapid (20–40 s) and also an AR-sensitive increase of intracellular Ca^{2+} after testosterone (0.3–3 μM) and 5α-dihydrotestosterone application was observed in isolated Sertoli cells, whereas estradiol had a small and progesterone no effect [69]. The effect on intracellular Ca^{2+} concentration was inhibited by the AR-antagonist hydroxyflutamide, the 5α-reductase inhibitor finasteride or by removal of extracellular Ca^{2+} (Ca^{2+}-depleted medium containing 2 mM EGTA) or pharmacological blockade of voltage-gated/non-voltage-gated Ca^{2+}-channels with verapamil and ruthenium red, respectively. These findings suggest that the androgen-induced rise of cytoplasmic Ca^{2+} involves sequential 5α-reduction, binding to a classical-AR, and activation of transmembrane influx of extracellular Ca^{2+}. Further, nilutamide could block the androgen effect on L-type Ca^{2+}-channels in guinea-pig cardiomyocytes [5]. In another study, testosterone (1–1000 pM) induced a rapid (20–30 s) increase of intracellular Ca^{2+} level in rat Sertoli cells and in a human prostatic cell line (PC3) [121], which was abolished by the non-steroidal AR-antagonists flutamide (1 μM) and finasteride (1 μM), but not by the classical, intracellular steroidal AR-antagonist cyproterone acetate (1 μM). This observation and the fact that these PC3 cells do not contain intracellular ARs, support the existence of alternative intracellular-ARs or transmembrane-ARs different from the classic-ARs [121]. All these results suggest, that the rapid AR-sensitive effects on intracellular Ca^{2+} concentration are predominantly mediated by the actions of classic-ARs [29,121,172,183].

6.2. AR-insensitive modulation of intracellular Ca^{2+}-hemostasis

Besides the AR-sensitive pathway an AR-insensitive pathway exists. Androstenedione (0.1 nM–1 μM) increased the intracellular Ca^{2+} level within seconds in cells from follicular fluids, an effect resulting from both transmembrane influx and mobilization of Ca^{2+} from ER [123]. Machelon et al. demonstrated, that under cotreatment conditions with the G-protein inhibitor PTX (100 ng/ml) and the specific PLC-inhibitor (U-73122, 5 μM) the rapid cytoplasmic Ca^{2+} effect was abolished [123]. The AR-antagonist hydroxyflutamide did not inhibit the androstenedione-induced intracellular Ca^{2+} rise. Moreover, treating cells with verapamil, a typical L-type Ca^{2+} channel antagonist also inhibited the increase of $[Ca^{2+}]_i$ induced by androstenedione, suggesting a rapid agonistic androgen effect on voltage-gated L-type Ca^{2+}-channels. These findings indicate that the intracellular mobilization of Ca^{2+} involves PLC activation and PTX-sensitive G-proteins [123]. Besides androstenedione, testosterone (10 pM–10 nM) also increased the intracellular Ca^{2+} transient rapidly within seconds in male rat osteoblasts [114]. In addition, IP_3 and diacylglycerol (DAG) synthesis was induced within seconds [114], which indicates the involvement of a GPCR → PLC route. In contrast, 17β-estradiol and progesterone, have no effect on intracellular calcium, indicating a specific action of androgens in male rat osteoblasts [114]. Interestingly, similar concentrations of testosterone did not elicit an increased in $[Ca^{2+}]_i$ in female rat osteoblasts, whereas 17β-estradiol increased $[Ca^{2+}]_i$ at concentrations as low as 1 pM [115], an effect not seen in male osteoblasts. These results confirm a sex-dependent rapid effect of androgens and estrogens in rat osteoblasts [114].

Testosterone (10–100 nM) or 19-nor-testosterone (100 nM) were able to produce a fast and transient increase of intracellular Ca^{2+} in skeletal muscle cells, whereas other steroids, like estradiol, progesterone, or dexamethasone had no effect on intracellular Ca^{2+} levels [52,53]. At 10 nM testosterone, approximately 40% of the cells

responded, whereas at 100 nM approximately 70% showed a response [52]. Furthermore, Estrada et al. demonstrated that this effect of testosterone is also mediated by membrane G-protein coupled receptors. Testosterone conjugated to bovine serum albumin, which preferentially targets membrane-ARs, produced an intracellular calcium release similar to that obtained with the free testosterone. The testosterone-induced intracellular Ca^{2+} release was partially abolished by a PLC inhibitor (U-73122, 10 μM) and completely by an IP_3-receptor blocker (xestospongin B, 40 μM), or by a G-protein inhibitor (PTX, 1 μg/ml) [52]. Pretreatment with an intracellular AR-antagonist (cyproterone, 1 μM), a modulator of RyR (ryanodine, 20 μM), and a tyrosine kinase inhibitor (genistein, 50 μM), did not modify the Ca^{2+} response, suggesting an AR-independent and PTX-sensitive G-protein/PLC/IP_3-mediated rapid androgen effect in myotubes [52]. The same pathway could be detected in neuroblastoma cells and rat cardiomyocytes [54,201]. In contrast, the androgen-induced Ca^{2+} influx in LNCaP cells seems to be regulated by a PLC-insensitive pathway [190]. Sun et al. showed a G-protein-linked mechanism induced by androgens, which resulted in an activation of L-type Ca^{2+} channels. The rapid response triggered by 5α-dihydrotestosterone was not mediated through intracellular-AR, c-Src kinase-AR complex, or SHBG-R, suggesting the existence of a novel membrane GPCR pathway [190].

All these results demonstrated that the androgen-induced increase in $[Ca^{2+}]_i$ cannot be blocked by AR-antagonists, which suggests a G-protein coupled receptor-mediated pathway (e.g., androgen binding to SHBG-R with induction of cAMP synthesis) or a direct androgen action on a target protein (e.g., androgen binding to the pore-forming subunit of voltage-gated Ca^{2+}-channels) [50,52,135]. Further, it was reported that hydroxyflutamide, an androgen analog and anti-androgen, exerted an AR-insensitive and non-genomic effect in AR-negative prostate cancer cells through the tyrosine kinase receptor EGFR [110]. Moreover, the non-genomic effect of androgens is able to exert genotropic actions by a membrane-AR/GPCR/PLC → $[Ca^{2+}]_i$/lipopolysaccharide (LPS) → MAPK → p38 kinase pathway [7,72]. These results indicate that there is a cross-talk of the testosterone-induced non-genomic Ca^{2+} signaling with the LPS-signaling route, independent from the intracellular-AR-mediated genomic pathways.

7. Rapid androgen effect on ion-channels

The concept that androgens can act rapidly and specifically on plasma membrane proteins, including voltage and ligand-gated ion-channels or G-protein-coupled receptors, is based on works with the neuroactive steroids [84,99,148].

7.1. Rapid androgen effect on ligand gated ion-channels

The steroids synthesized by the brain and nervous system, given the name neurosteroids, have a wide variety of diverse functions. The major groups of neuroactive steroids, and their metabolites, are progesterone, deoxycorticosterone, and some androgens, notably dihydroxyepiandrosterone [1,220]. In general, they mediate their actions, not through classic steroid receptors, but through other mechanisms such as ligand/neurotransmitter-gated channels, and direct or indirect modulation of other neurotransmitter receptors [131]. One of the major targets of these neurosteroids are ligand-gated ion-channels including $GABA_A$, glutamate or opioid receptors [2,34,35,84,94,206]. Neurosteroids and also androgens rapidly stimulated the MAPK kinase and PI-3K/Akt pathway or directly modulated ion-channels in astrocytes and neurons [1,129,184,216].

Especially for the $GABA_A$ receptor it is known that neuroactive steroids interact specifically at a site of the receptor complex distinct from the benzodiazepine and barbiturate modulatory sites [136]. The first evidence that steroids could regulate the $GABA_A$ receptor were derived from studies demonstrating that the steroidal anesthetic, alfaxalone, potentiated GABA effects and enhanced the binding of GABA ligands [125]. Subsequent studies extended these findings to the endogenous steroid androsterone by demonstrating that it also potently potentiated GABA effects and increased the binding of GABA ligands [77]. Recently, Hossie et al. identified two discrete binding sites in transmembrane domains of the $GABA_A$ receptor that mediate the potentiating and direct activation effects of neurosteroids [84]. The study showed that the activation and potentiation of $GABA_A$ receptors by neurosteroids are mediated by two discrete groups of residues in the $GABA_A$ receptor transmembrane domains [84]. Activation of the $GABA_A$ receptor complex by such neurosteroids results in opening of its central Cl^--conducting pore, which led to a hyperpolarization of the plasma membrane and inhibition of neuronal firing. The $GABA_A$ receptor agonist-like effects of these neuroactive steroids are similar, but not identical, to those of benzodiazepines and barbiturates [80]. The endogenous steroids are approximately 10 times more potent than benzodiazepines and 200 times more potent than pentobarbital in potentiating GABA-mediated Cl^--uptake in rat cerebral cortical synaptoneurosomes [140]. Reduction of testosterone by 5α-reductase to 5α-dihydrotestosterone, which is then converted to 3α-androstanediol (3α-diol), provide a powerful $GABA_A$ receptor modulating neurosteroid with anticonvulsant properties. Both 5α-reduced metabolites of testosterone had a powerful anti-convulsant activity [157]. 3α-androstanediol protected mice against seizures induced by $GABA_A$ receptor antagonists (e.g., picrotoxin) in a dose-dependent manner [156]. The anxiolytic, anti-convulsant and sedative-hypnotic actions of these neuroactive steroids have resulted in their use as therapeutic agents for treatment of anxiety, epilepsy, insomnia, and possibly for the alteration of pain thresholds [2,58,220].

7.2. Rapid androgen effect on voltage gated ion-channels

Androgens are also able to modulate voltage-gated ion-channels [5,50,52,117,135]. It is known that women with

virilization exhibit a shorter and faster repolarization time than normal women and castrated men, suggesting that testosterone is an important modulator of cardiac ventricular repolarization [13]. Many experimental studies about the long-term effect of androgens observed a change in mRNA and protein levels, but only few studies analyzed the functional rapid effect of androgens on voltage-gated ion-channels.

To explore which voltage-dependent ion-channels are targets of testosterone, Bai et al. examined the action of testosterone (100 nM) in guinea pig cardiomyocytes [5]. Testosterone (100 nM) suppressed L-type Ca^{2+}-current ($I_{Ca,L}$) and enhanced the slow delayed rectifier K^+-current (I_{Ks}), whereas the effect on the rapid component (I_{Kr}) was negligible. The testosterone-induced changes of cardiac ion-current densities were completely inhibited by the AR-antagonist nilutamide (1 μM). Additional analysis demonstrated that the testosterone effect on these ion-channels was mediated by an activation of AR → c-Src → PI-3K/Akt → NOS3 pathway [5]. A number of studies extended these observations and demonstrated a modulation of further voltage-gated ion-channels by androgens [5,31,51,83,176]. Testosterone relaxed smooth muscle by opening large-conductance, Ca^{2+}- and voltage-activated K^+-channels (BK_{Ca}) [41] and ATP-sensitive K^+-channels (K_{ATP}) [31,83,87,176], in addition to inhibition of voltage-gated Ca^{2+}-channels [37,50,73,135,149,150,173]. The effect of testosterone on BK_{Ca}-channel activity was mimicked by the cell-permeable cGMP analog 8-bromo-guanosine 3′,5′-cyclic monophosphate (8-bromo-cGMP), which suggests a cGMP dependent pathway. Moreover, several observations support a non-genomic modulation of K_{ATP}-channels by testosterone [31,83,87,176]. Sarcolemmal K_{ATP}-channels are heterooctamers composed of two disparate subunits, a two transmembrane helix pore forming subunit Kir6.x, and its regulatory subunit, the ATP-binding cassette (ABC) protein SUR (sulfonylurea receptor). Kir6.x subunits are members of the K^+ inward rectifier channel family, that in physiological solutions conduct K^+ better in inward than in outward direction. These channels are weakly inwardly rectifying K^+-channels that stabilize the membrane close to the equilibrium potential for K^+, thereby counteracting membrane depolarization. A recent study demonstrated that testosterone (0.1–300 μM) induced a rapid vasorelaxation in isolated human radial artery by opening vascular K_{ATP}-channels [176]. Importantly, this testosterone-induced effect does not involve the cyclooxgenase or nitric oxide-pathway, and was not mediated by BK_{Ca} and K_v-channels. In cardiomyocytes, K_{ATP}-channels have been identified both in the sarcolemmal (sarc-K_{ATP}) and in the mitochondrial inner membrane (mito-K_{ATP}). Opening of mito-K_{ATP}-channels has been demonstrated to protect cardiomyocytes against hypoxic cell death [51]. One study showed an androgen-induced cell protection via direct modulation of cardiac mito-K_{ATP}-channels by testosterone, whereas testosterone exhibited no effect on sarc-K_{ATP} channels [51]. In contrast, testosterone (0.1–10 μM) inhibited sarc-K_{ATP}-channels in rat Sertoli cells [202], suggesting a tissue dependent modulation of testosterone on sarc-K_{ATP}-channels. In addition to K_{ATP}-channel regulation by nucleotide (e.g., ATP) concentrations, the channel activity is also dependent on the amount of membrane phospholipids, including PIP_2 and PIP_3. It has been suggested that PIPs and ATP bind to overlapping sites of the K_{ATP}-channel [122]. The levels of PIP_2 in the cell membrane may determine the basal activity of the K_{ATP}-channel ($[PIP_2]\downarrow \rightarrow [K_{ATP}\text{-activity}]\downarrow$). Those observations indicate, that the channel activity would be modulated by a G-protein coupled pathway (GPCR → PLC → PIP_2), which influences the membrane pool of PIP_2. One study demonstrated that testosterone depolarises the membrane potential, augments resistance and Ca^{2+}-uptake in Sertoli cells of seminiferous tubules, whereas these actions were abolished in the presence of the K_{ATP}-channel opener diazoxide [118]. The action of testosterone on K_{ATP}-channels was completely blocked by addition of the PLC inhibitor U73122 [118], indicating that the regulation of the K_{ATP}-channel results from the PIP_2 depletion of the cell membrane by testosterone (testosterone → GPCR → PLC → $[PIP_2]\downarrow \rightarrow [K_{ATP}\text{-activity}]\downarrow$ → depolarization → activity of L-type Ca^{2+}-channel ↑) [89,118]. Other possible mechanisms include a direct modulation of the ion-channel conducting pore (e.g., androgen-binding site) or indirect regulation by second messengers or phosphorylation.

Among second messenger systems, Ca^{2+} is one of the most potent mediators. The intracellular Ca^{2+} concentration is regulated by Ca^{2+} influx (L- or T-type Ca^{2+}-channels) and Ca^{2+} efflux through cell membrane (e.g., Na^+/Ca^{2+} exchanger and Ca^{2+}-ATPase) and by intracellular Ca^{2+} stores of the ER (e.g., RyR and IP_3-receptors). Androgens are able to modulate all these systems [47,50,52,91,114,135,190]. Testosterone (10 pM–10 nM) increased the intracellular Ca^{2+} concentration in osteoblasts within 5 s via Ca^{2+} influx through voltage-gated Ca^{2+}-channels and via Ca^{2+} mobilisation from the ER as shown by the effect of thapsigargin. Testosterone also increases IP_3 an DAG formation within 10 s, which suggest a GPCR → PL → PIP_2 → IP_3/DAG mediated pathway [114]. In contrast to this study in rat osteoblasts, testosterone (10–100 nM) led to a rapid inhibition of voltage-gated Ca^{2+}-channels in rat cardiomyocytes (10–100 nM) [50,135]. Flutamide (10 μM) did not prevent the testosterone-induced inhibition of these cardiac Ca^{2+}-channels, whereas nilutamide could block the androgen effect on L-type Ca^{2+}-channels in guinea-pig cardiomyocyte [5], suggesting a species-specific effect of androgens. The lipophilic testosterone may act like a dihydropyridine derivate on the Ca^{2+}-channel ion-pore through the lipid phase of the cell membrane. Because of their very poor water solubility, it is unlikely that testosterone enters into the Ca^{2+}-channel from the cytoplasm. Therefore testosterone may bind directly to the pore-forming subunit of the Ca^{2+}-channel. Two studies

confirmed that epiandrosterone and testosterone act as a L-type Ca^{2+}-channel antagonist with properties similar to those of dihydropyridine Ca^{2+}-channel blockers, implying that androgens could serve as "endogenous dihydropyridines" [73,75]. In contrast to these results concerning L-type Ca^{2+}-channels, one study demonstrated that testosterone enhances Ca^{2+} transport within 5 min by opening T-type Ca^{2+}-channels via a MAPK and tyrosine kinase pathway [37].

8. Rapid androgen effect on plasmalemmal ion-tansporters

Among all second messenger systems, Ca^{2+} is the most potent molecule in triggering neurotransmission. Impairment in both intracellular Ca^{2+} concentration and Ca^{2+} signaling reduces neuronal activity. The increase in $[Ca^{2+}]_i$ due to Ca^{2+} influx through plasma membrane Ca^{2+} channels or by the release from intracellular compartments can be reduced by Ca^{2+} efflux via plasma membrane or by Ca^{2+} sequestration into intracellular stores. In excitable tissues the plasma membrane contains two systems responsible for the extrusion of Ca^{2+}: the Na^+/Ca^{2+} exchanger and the Ca^{2+}-ATPase. Steroids are able to interact with this transmembrane transporter and enzyme.

Several studies support that these plasma membrane Ca^{2+} pumps might be possible membrane targets for a non-genomic androgen action [64,221]. Androgens directly modulated the activity of plasma membrane Ca^{2+}-ATPase purified from synaptosomal membranes of rat cortex [221]. The activity of Ca^{2+}-ATPase was analyzed after a short-time (60 s) preincubation under different concentrations of pregnenolone, dehydroepiandrosterone sulfate, testosterone and 17β-estradiol. Testosterone affected Ca^{2+}-ATPase activity in a gradual manner and 17β-estradiol provided no further enhancement of the enzyme, suggesting a structure-specific action of sex hormones. Another study in synaptosomal membranes of dog brain demonstrated that testosterone increased the activity of Ca^{2+}-ATPase, whereas progesterone revealed an opposite effect [42]. Further, testosterone treatment induced a maximum increase in Na^+/Ca^{2+}-exchanger mRNA levels, however studies analyzing the rapid, non-genomic effect are still lacking [68].

Another membrane effector, Na^+–K^+-ATPase, was modulated rapidly by different sex hormones [64]. An interaction of androgens with the Na^+–K^+-pump could be observed in several studies [57,64,168,174]. For example, the Na^+–K^+-ATPase pump, was significantly inhibited in estradiol- and progesterone-treated cells, but significantly stimulated by testosterone treatment [64].

9. Physiological and clinical relevance of rapid androgen action

A major question about the rapid effects of androgens is whether there is a physiological role and clinical relevance on different systems, like on the cardiovascular system or on neurons. Sex hormones have emerged as important modulators of cardiovascular physiology and pathophysiology with many factors being involved. Cardiomyocytes have been shown to be androgen targets [68,130,187]. In men, the risk of developing coronary artery disease (CAD) and hypertension is much higher than in premenopausal women [100,134,210]. However, cardiovascular protection during the reproductive age is lost after menopause [182]. These observations led to the hypothesis that estrogens are beneficial and androgens are detrimental to the cardiovascular system. Increasing evidence indicates that estrogen is an important cardiovascular protective factor not only by means of long-term effects, but also by means of direct non-genomic actions on vascular structure and function [30,178]. Therefore, it was believed that estrogen treatment in post-menopausal women would be cardioprotective. However, two large randomised controlled trials of female hormone replacement were disappointing not showing any beneficial cardiovascular effect [86,166]. Numerous clinical and epidemiological studies reported a controversial relationship between androgens and cardiovascular disease [74,196]. In all these studies it remains difficult to distinguish between genomic and non-genomic androgen effects. Men with CAD have significant lower levels of testosterone than controls, thus implying that low circulating testosterone levels in men are correlated with progression of atherosclerosis [48,49,101,127,128]. Indeed, numerous risk factors for CAD are associated with hypotestosteronaemia [85,127]. Consistent with these findings, testosterone replacement therapy improves myocardial ischemia in patients with CAD, an effect presumably due to testosterone-induced coronary vasodilatation, and improves insulin resistance, cholesterol and visceral adiposity, which represents an overall reduction in cardiovascular risk factors [3,44,48,76,96,161]. Chronic heart failure due to idiopathic dilated cardiomyopathy is also associated with a significant decrease in testosterone level [105]. Androgens have also been shown to exert-specific vascular effects. Rabbit coronary arteries treated with testosterone (1–100 μM) showed a direct, non-genomic endothelium-independent relaxation, which clinically might be beneficial [214]. Acute intracoronary or intravenous infusion of testosterone provides rapid improvements in myocardial ischemia [92,161,203]. Further, an acute administration of buccal testosterone immediately increased cardiac output, apparently via reduction of left ventricular afterload [155]. These studies confirm a rapid—mainly vasodilatory effect—of testosterone, which is thought to underlie the potential beneficial effect of androgens on the cardiovascular system. Although testosterone long-term treatment in supra-physiological concentrations causes myocardial hypertrophy [97], studies of androgen replacement therapy in physiological level in the elderly had no obvious effect on cardiovascular morbidity and mortality. Currently available data on acute androgen actions on the cardiovascular system are promising, however, still very limited [126,161,193,203]. Further studies are clearly warranted.

In neurons, testosterone induced an increase of cytosolic and nuclear Ca^{2+} level, which is a critical mechanism for

controlling neurite outgrowth [54]. At the cytosolic level, Ca^{2+} increases the binding of androgens to their receptor [21]. Further, androgens are able to activate Ca^{2+}-dependent kinase pathways, such as Erk, PI-3K or Src [52,137], which may phosphorylate the AR and enhance its activity. Moreover, it has been suggested that both the temporal and spatial changes of the nuclear [111] and cytosolic Ca^{2+} signal [43,113] are involved in controlling gene expression, which suggests an interlink between genomic and non-genomic pathways. Androgens are also able to rapidly modulate neuronal synaptic plasticity [141]. Neuropsychiatrically testosterone treatment was associated with significant improvement in mood [128], whereas a potential risk for patients with bipolar disorder is being discussed. Further, some studies demonstrated that androgens affect learning and memory [46]. A rapidly anxiolytic effect of testosterone could be shown in mice [2]. Future research and clinical studies will be required to determine if these experimental observations can be translated to the clinical setting and may have any therapeutic relevance.

10. Conclusion

The rapid actions of androgens are mediated by direct binding to the target protein (e.g., ion-channel) or by a specific association to different receptors. The non-genomic androgen action based on receptor level can be mediated by at least three androgen-binding proteins, the classical intracellular androgen receptor, the transmembrane androgen receptor and the transmembrane sex hormone-binding globulin receptor. For both transmembrane receptors, the non-genomic effect is converted via a G-protein coupled process, whereas binding to intracellular androgen receptors may lead to an activation of several cytosolic pathways. All rapid androgen actions are predominantly mediated by second messenger signaling (especially Ca^{2+}) and phosphorylation events, including different intracellular signal routes, e.g., PKA, PLC-PIP_2, MAPK, PI-3K, Src or PTX-sensitive G-protein/PLC/IP_3-pathways. Although some studies implicated benefits of the non-genomic androgen actions on the cardiovascular and neuropsychiatric systems, more detailed research and clinical studies are still required.

Acknowledgments

Supported by grants from the Deutsche Forschungsgemeinschaft (MI 960/1-1, Ho 2146/3-1), the Koeln Fortune Program/Faculty of Medicine/University of Cologne, and the Marga & Walter Boll-Stiftung (Germany).

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