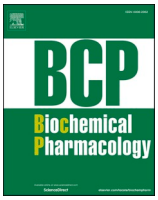
Biochemical Pharmacology 203 (2022) 115200

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm



Review

# Androgens and Non-Genomic vascular responses in hypertension

Mercedes Perusquía *

*Departamento de Biología Celular y Fisiología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, 04510 Ciudad de México, México*

ARTICLE INFO

*Keywords:*
Androgens
Male sex steroids
Androgen-induced vasorelaxation
Androgen-induced hypotension
Antihypertensive responses
Blood pressure regulation

ABSTRACT

Arterial hypertension is a global public health concern. In the last few years, the interest in androgen deficiency has been growing, and the association between androgens and high blood pressure (BP) is still controversial. One purpose of this review was to summarize the available findings in order to clarify whether male sex steroid hormones have beneficial or harmful effect on BP. The second purpose was to enhance the recognition of the acute non-genomic sex–independent vasorelaxing effect of androgens. Remarkably, BP variation is expected to be a consequence of the androgen-induced vasorelaxation which reduces systemic BP; hence the *in vivo* vasodepressor, hypotensive, and antihypertensive responses of androgens were also analyzed. This article reviews the current understanding of the physiological regulation of vascular smooth muscle contractility by androgens. Additionally, it summarizes older and more recent data on androgens, and some of the possible underlying mechanisms of relaxation, structural–functional differences in the androgen molecules, and their designing ability to induce vasorelaxation. The clinical relevance of these findings in terms of designing future therapeutics mainly the 5-reduced metabolite of testosterone, 5β-dihydrotestosterone, is also highlighted. Literature collected through a PubMed database search, as well as our experimental work, was used for the present review.

## 1. Introduction

### 1.1. General considerations of androgens

Steroids are important in biology, chemistry, and medicine. These compounds are divided in two different classes: natural or synthetic biologically active organic compounds, with a molecular structure of 17 carbon atoms arranged in four fused rings (A, B, C, and D) and called "cyclopentenoperhydrophenanthrene" They are found in plants, animals and fungi, and they regulate several biological functions.

Sex steroid hormones can be grouped into three families based on their number of carbon atoms: pregnanes (21), androstanes (19), and estranes (18). Therefore, male sex steroids (androgens) are also classified as C19-steroids which are traditionally considered to maintain male sexual characteristics, although their relevant biological roles in the bone, muscle, prostate, and adipose tissue and the reproductive, cardiovascular, immune, neural and hematopoietic systems have also been documented [1,2]. In this review, it has been highlighted the physiological effects of C19-steroids (androgens) on the cardiovascular system which have previously been scarcely considered.

It is important to take into account the fact that androgens are metabolized into different steroids each with their own variable effects and potency. Fig. 1 summarizes the classical gonadal pathway biosynthesis of androgens and the enzymes involved therein. In males, the testes synthesize testosterone (TES) from dehydroepiandrosterone (DHEA) and androstenedione, which is then mainly converted into 5α- and 5β-dihydrotestosterone (5α-DHT and 5β-DHT, respectively) in target tissues, such as the prostate and seminal vesicles. In females, TES produced from the theca cells is predominantly converted into estrogens in the granulosa cells of the ovary (Fig. 1) (for details, see [3]). The major androgens produced by adult humans in decreasing order of magnitude are DHEA, androstenedione, TES and 5α- and 5β-DHT. It is important to emphasize that androgens are not exclusive to males and estrogens are not exclusive to females; both sexes, male and female,

*Abbreviations:* 5α-DHT, 5α-dihydrotestosterone; 5β-DHT, 5β-dihydrotestosterone; AAS, anabolic–androgenic steroids; AR, androgen receptor; ARE, androgen response element; $BK_{Ca}$, large-conductance $Ca^{2+}$-activated $K^+$ channels; MAP, mean arterial pressure; BP, blood pressure; BSA, bovine serum albumin; cAMP, cyclic adenosine monophosphate; CVD, cardiovascular disease; DHEA, dehydroepiandrosterone; $K_{ATP}$, ATP-sensitive $K^+$ channels; $K_{IR}$, inward-rectifier $K^+$ channels; $K_V$, voltage sensitive $K^+$ channels; L-NAME, $N^{\omega}$-nitro-L-arginine methyl ester; L-VOCCs, L–voltage-operated $Ca^{2+}$ channels; NO, nitric oxide; ROCC, receptor-operated $Ca^{2+}$ channels; SHR, spontaneously hypertensive male rats; SM, smooth muscles; SOCE, store operated $Ca^{2+}$ entry; TES, testosterone; Tfm, testicular feminized male; VSM, vascular smooth muscles; WKY, Wistar Kyoto.

* Corresponding author at: Instituto de Investigaciones Biomédicas, PO Box 70228, Ciudad Universitaria, 04510, Ciudad de México, México.
*E-mail address:* perusqui@unam.mx.

https://doi.org/10.1016/j.bcp.2022.115200
Received 10 June 2022; Received in revised form 27 July 2022; Accepted 28 July 2022
Available online 1 August 2022

produce androgens and estrogens but their proportions are different. Thus, the plasma concentration of TES in males is 10–35 nM [4] and 0.7–2 nM in females [5].

However, little attention has been paid to the metabolism and changes in the chemical structure of androgens. TES is reduced to 5α-DHT, which is considered the most potent androgen [6–9]; however, its 5β-reduced isomer (5β-DHT) has been mistakenly considered biologically inactive which will be discussed later.

### 1.2. Genomic vs non-genomic mechanisms of androgen action

Androgens may modify cellular function through both (i) their well-known classical genomic action and (ii) their more recently identified non-genomic mechanisms of action.

The genomic mechanism of action has been extensively studied. It regulates many of the most known biological functions such as secondary male sexual characteristics, sexual behavior, spermatogenesis, increment of muscle mass, loss of adipose tissue, prostate growth, prevention of osteoporosis, and roles in the hematopoietic, immune, and nervous systems, and in skin hair follicles, emotions, and libido [1,2]. Classically, androgens may mediate their biological responses by binding to the androgen receptor (AR). ARs are expressed in many tissues, with the highest concentrations occurring in male reproductive organs [10]. Briefly, the AR signaling pathway for genomic action signaling involves androgens crossing the plasma membrane, entering the cytoplasm and binding to the AR (androgen-receptor complex), resulting in the dissociation of chaperone proteins and translocation of the complex to the nucleus where it dimerizes and binds to the androgen response element (ARE) for modulating gene transcription, and leads to protein synthesis creating a biological response, which has been extensively studied (for review see [11–13]). This classical androgen response is relatively slow, occurring over a period of hours, weeks, or years (Fig. 2).

In contrast to the genomic pathway, the non-genomic mechanism of action began to be recognized much later, roughly 40 years ago. Different steroids, including androgens, can also act via this mechanism, which is independent of the ligand-dependent transactivation function of nuclear receptors [14,15]. This is known as 'non-genomic' signaling, which typically occurs within a short time frame (seconds to minutes) (Fig. 2) [16,17]. Fig. 2 summarizes the two mechanisms of androgen action.

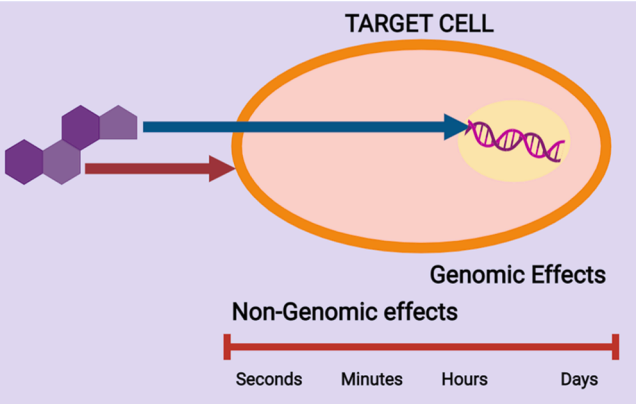



**Fig. 2. Differences of sex steroid mechanisms of action.** Non-genomic mechanism (red arrow): androgen responses occur through membrane which are independent of the ligand-dependent transactivation function of nuclear receptors, they are rapid onset (seconds to minutes), short duration, not modified by inhibitors of transcription or protein synthesis. Genomic effects (blue arrow): the androgen receptor signaling pathway for genomic action signaling involves androgens crossing the plasma membrane, entering the cytoplasm and binding to the androgen receptor (androgen-receptor complex), resulting in the translocation of the complex to the nucleus where it dimerizes and binds to the androgen response element for modulating gene transcription, and leads to protein synthesis creating a biological response which are slower onset (minutes to days), and longer duration.

The effects mediated by the non-genomic type of action have been observed in most excitable tissues, such as the central nervous system and reproductive and non-reproductive smooth muscles, among others. Several studies have found that the majority of the physiological non-genomic androgen responses occur through membrane- or membrane-associated AR/binding proteins, which would decrease of cell membrane fluidity, changes in intracellular calcium concentration ($[Ca^{2+}]_i$), activation of the second messenger pathway, or a combination of the above mechanisms (reviewed in [18–21]). Notably, non-genomic mechanisms can act independently or be associated with other genomic effects in order to elicit a physiological response, *i.e.*, the genomic and non-genomic pathways of androgen action are likely inter-

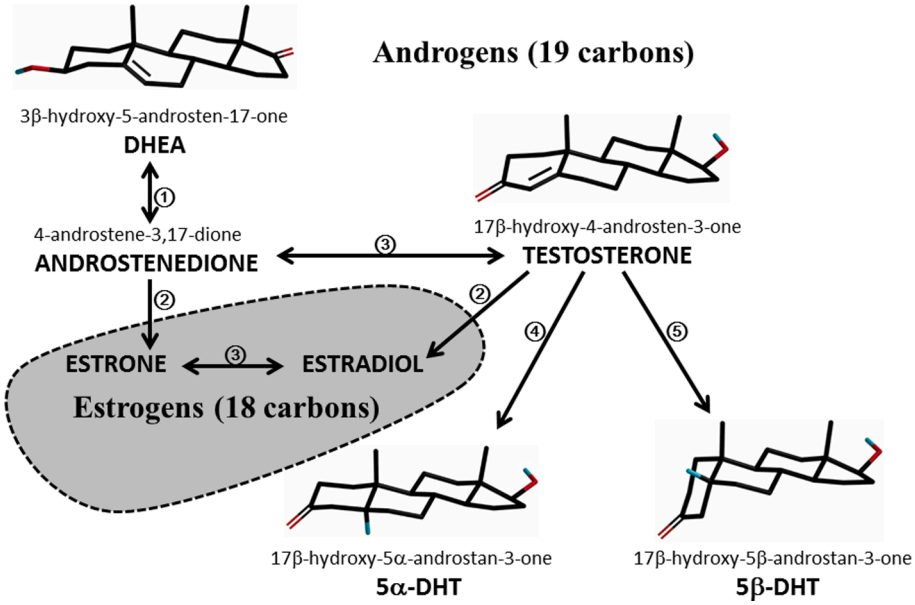



**Fig. 1. Classical pathway biosynthesis of androgens (C19-steroids) and estrogens (C18–steroids).** Enzymes involved: 1) 3β-HSD, 3β-hydroxysteroid dehydrogenase/D5-4 isomerase; 2) Aromatase; 3) 17β-HSD, 17β-hydroxysteroid dehydrogenase; 4) 5α-reductase; 5) 5β-reductase.

linked.

### 1.3. Classical effects of androgens

#### 1.3.1. Reproductive effects

As mentioned before, the major biological actions of TES include the development and maintenance of genetically mediated secondary sexual characteristics in males. These effects are mediated directly by TES and its two major active metabolites, 5α-DHT and 17β-estradiol. The genomic action, mediated by AR, of TES and 5α-DHT is responsible for sexual differentiation and virilization. Likewise, these androgens play an important role in the deepening of the voice, increase in sebum production, and in the enlargement of the external genitalia, including penile length. The conversion of TES to 5α-DHT, but not to 5β-DHT, in the prostate is essential for its growth and development, and, importantly the concentration of this 5α-reduced metabolite is 10 times higher than that of TES. Similarly, erectile dysfunction, hypogonadism, and metabolic syndrome, have all been associated with TES deficiency. In addition to the masculinizing effects of androgens, they also have anabolic properties. TES is known for producing muscle hypertrophy by increasing fractional muscle protein synthesis [22,23]. The correct term for these steroids is anabolic–androgenic steroids (AAS): "anabolic" refers to muscle building and "androgenic" refers to increased male sex characteristics. AAS stimulates growth in many types of tissues, especially bone and muscle, and their effects involve binding to the AR and therefore genomically mediated. AAS have been used since the 1950 s in pharmacological doses (administered orally and by several parenteral ways) in an attempt to maximize the well-known anabolic effects of TES, reduce the rate of its hepatic inactivation, and decrease its aromatization to 17β–estradiol [24]. AAS as TES derivates and analogs include the endogenous sex male steroids i.e., TES and 5α-DHT, as well as other agents and synthetics that behave like these androgens, some of which are nandrolone, decanoate, oxandrolone, oxymetholone, stanozol, TES cypionate, and TES enanthate.

#### 1.3.2. Effects of androgens on reproductive and non-reproductive smooth muscles

It has been demonstrated that androgens target reproductive and non-reproductive smooth muscles (SM) by inducing non-genomically mediated relaxation.

In urogenital SM, the uterine contractility is notably relaxed by androgens with a higher utero-relaxant potency than progestins [25–27]. Therefore, the uterus is a target organ for androgens that induce myometrial quiescence, which plays a key functional role in the maintenance of normal gestation. Androgens also lead to marked relaxation of barium-induced contraction of the rat epididymis and seminal vesicles [28]. The relaxing effects of TES were also confirmed in rabbit seminal vesicles [29]. The neck of the urinary bladder is also a target of androgen-induced relaxation; thus, TES and 5α-DHT have been found to elicit similar concentration-dependent relaxation in both male and female pigs [30,31]. In summary, the acute relaxing effect of androgens in other types of male urogenital SM has rarely been studied.

In gastrointestinal SM, progesterone, 17β-estradiol, and TES are also capable of inhibiting the contractibility of intestinal SMs such as of those in the ileum [32,33]. In comparison with female steroids, the high potency of androgens in inducing non-genomic gastrointestinal relaxation was recognized [33,34]. TES and 5α-DHT have also been found to be capable of inducing similar levels of relaxation in gallbladder SMs in male guinea pigs [34]. Similarly, several androgens (DHEA, TES, and its 5-reduced metabolites) are capable of inducing non-genomic bronchorelaxation by acting on the airway SMs as well as inducing genomic anti-inflammatory responses, suggesting a potential therapeutic use in the treatment of severe asthma (reviewed in [35]). A substantial point of interest in this review is that vascular SM (VSM) is also a target of TES and its metabolites in acute non-genomic relaxation. In particular, in the last few years, there has been a growing interest in androgen-induced vasorelaxation, which is discussed in detail in Section 2.

Therefore, the available data indicate that multiple types of SM are targets for androgen–induced relaxation, and that androgens may be important in the regulation of SM excitability in general.

## 2. Vascular effects of androgens

### 2.1. Vasorelaxation/vasodilation of isolated vessel studies

It has been widely accepted that estrogens exert protective effects on female vasculature [36,37]. Recent clinical and experimental animal studies have revealed that TES, the primary and most well studied androgen, directly protects against risk factors for cardiovascular disease (CVD), such as atherosclerosis and hypertension, and reduces the incidence of CVD, such as cardiac failure and myocardial ischemia [38–44]. Likewise, several clinical trials have pointed out that androgen deficiency seems to exacerbate many risk factors and pathologies associated with cardiovascular and metabolic diseases [41,45–50]. Based on these findings, the protective effect of TES on the cardiovascular system is gaining recognition. In this context, the main purpose of the present review article is to show the direct and rapid non-genomic effects at the vascular level, particularly in the regulation of blood pressure (BP).

Sex steroid hormones, including androgens, mediate vasorelaxation. Particularly regarding androgens, in the last 25 years, there has been a plethora of evidence that TES is capable of inducing vasorelaxation. The acute vasorelaxing effect of TES on contractions induced by depolarization, or several agonists in a number of isolated vascular beds of various species of mammals including humans (revised in [18,20,21,51–54]).

Notably, the acute concentration-dependent vasorelaxing effects of androgens have been reported in isolated human blood vessels, including the internal mammary artery [55], umbilical arteries [51,56], and pulmonary arteries and veins [57]. TES and 5α-DHT have also been reported to induce vasodilating effects on the penile and cavernosal arteries [58–60], and internal spermatic veins [61], the lack of vasodilation may cause non-psychogenic erectile dysfunction associated with hypoandrogenism (androgen deficiency). Moreover, androgens have been shown to regulate prostatic blood flow [62]. As reviewed by Traish et al. [63], TES–propionate has been used for the last six decades to treat sexual issues to augment penile vasodilation.

TES has direct effects on vascular tone causing vasodilatation, and an increase in coronary blood flow [42,43]; thus, it has been observed that the coronary circulation is more sensitive to the vasorelaxant effect of TES than large vessels with a benefit on time to cardiac ischemia. Notably, clinical studies have highlighted beneficial effects of TES therapy on exercise-induced cardiac ischemia, an effect mediated by coronary vasodilation [64], which persists for 3 months and indeed 12 months [65].

Together, these findings reveal that TES exerts beneficial effects on the cardiovascular system by reducing vascular tone, which involve direct vasodilatory action on the peripheral vasculature and reduction of BP. For their acute and marked vasorelaxing effects, the male sex steroids have been named "vasoactive androgens".

### 2.2. Systemic hypotensive actions

The vasorelaxing effect induced by androgens implies an increase in blood flow, and therefore, a reduction in BP.

High blood pressure (hypertension), sometimes called "the silent killer," is a serious medical condition and a global public health problem. Hypertension is very common in older people and is a major health concern. The pathogenesis of essential hypertension, the most common type of hypertension, is multifactorial and highly complex. Factors that play an important role in this pathology include genetics, activation of the neurohormonal systems, such as the sympathetic nervous system and renin-angiotensin-aldosterone system, obesity, and increased

dietary salt intake. However, the etiology of hypertension remains unknown.

Sex steroid hormones have also been associated with hypertension, however, there is a polemic regarding whether female sex steroids are beneficial and male sex steroids are detrimental in BP. Male sex is considered a major contributing risk for CVD. A direct effect of androgens on promoting hypertension has been hypothesized [66], and was attributed to the myths that TES is capable of inducing adverse effects on the cardiovascular system. One of these effects is an increase in BP.

The theory of increased hypertension risk in response to TES remains largely unsubstantiated. In view of the non-genomic vasorelaxing properties of androgens, it is difficult to consider that TES can be detrimental to the cardiovascular system, since TES, the most important male sex endogenous hormone, plays a vital role in the regulation of biological processes in males. This review aims to clarify the reasons for this uncertainty.

Several lines of evidence, contradict the traditional view of the adverse effects of androgens on BP. The first evidence that androgens are associated with the regulation of BP through BP reduction was collaterally observed in classic studies by Selye, when the anesthetic effect of steroids was reported in the early 1940 s [67,68]. Later, a body of information associated low circulating levels of TES with cardiovascular dysfunction, particularly with hypertension [43,69–72]. This finding was confirmed in several reports indicating that low concentrations of TES in men are associated with higher BP [73–76]. Other studies have suggested that a low TES concentration in men may be a novel risk factor for hypertension or CVD. An important clinical study documented that acute administration of TES in humans with cardiac failure decreases peripheral vascular resistance and improves cardiac output [77]. An observational study in men showed that long-term TES therapy, for up to 8 years, produced marked and sustained gradual decreases in systolic and diastolic BP [78]. One recent study showed that hypertensive men had lower levels of free TES, and bioavailable TES, compared to normotensive men [79].

More recently, we reported important *in vivo* experimental research on animals. It is important to highlight that the consequence of androgen-induced vasorelaxation, widely discussed in the present review, is a decrease in BP. In this regard, numerous studies have suggested that low BP is linked to androgens (reviewed in [54]). We first reported that some TES metabolites are capable of blocking vasopressor responses to noradrenaline or a $Ca^{2+}$ channel agonist (Bay K 8644) in anesthetized vagosympathectomized, pithed, Wistar male rats [80]. Our recent *in vivo* experimental research has also demonstrated that BP regulation is an expected consequence of androgen-induced vasorelaxation [81–84]. We documented that TES and its 5β–reduced metabolite (5β-DHT) induced a marked reduction in BP in both conscious, normotensive, Sprague-Dawley rats and testicular feminized male rats (Tfm; androgen receptor–deficient) [81]. Afterwards, we reported that androgens can also elicit a significant antihypertensive response in conscious, spontaneously hypertensive, male rats (SHR) and in normotensive WKY rats who underwent TES deprivation by orchiectomy for inducing hypertension, which was then prevented by TES replacement therapy [82]. Similarly, it was demonstrated that in an *in vivo* rat model of preeclampsia, the elevated mean arterial BP (MAP) was reduced significantly by an intravenously bolus injection of DHEA, TES or its 5-reduced metabolites (5α- and 5β-DHT) [83]; notably, this study indicates that androgens are also antihypertensive in females. More recently, a model for studying androgen deficiency in conscious hypertensive rats caused by orchiectomy helped us to understand the progression of hypertension in aging men, since our experiments clearly demonstrated that hypertension is a disease associated with aging. We also observed that the intravenously bolus administration of DHEA, TES, or 5β-DHT is capable of inducing antihypertensive responses in hypertensive aging rats [84]. The above information is summarized in Table 1.

Notably, TES is an acute vasorelaxant in human subcutaneous resistance arteries [85]. In addition, functional studies have also shown that the vasorelaxing potency of 5β–DHT is higher in the mesenteric artery than in the thoracic aorta of hypertensive and normotensive rats [86]. These findings suggested that TES and particularly 5β-DHT regulate vascular resistance in the mesenteric arterial bed, explaining its outstanding antihypertensive response.

**Table 1**
*In vivo* experimental findings of androgen-induced hypotension and antihypertension.

| ANDROGENS | RESPONSES PRODUCED |
|---|---|
| 5α-, 5β-DHT and its 3α,5β-reduced metabolite | Antagonism of vasopressor responses to noradrenaline or $Ca^{2+}$ channel agonist in vagosympathectomized pithed rats [76] |
| TES, 5β-DHT | BP reduction (hypotension) in normotensive normal and Tfm rats [77] |
| TES, 5α- and 5β-DHT | Antihypertensive responses in SHR and ORX WKY rats [78] |
| DHEA, TES, 5α- and 5β-DHT | Antihypertensive responses in gestacional hypertension (preeclamtic rats) [79] |
| DHEA, TES, 5β-DHT | Antihypertensive responses in elevated blood pressure by androgen deficiency in ORX rats [80] |

DHEA (dehydroepiandrosterone), TES (testosterone), 5α-DHT (5α-dihydrotestosterone), 5β-DHT (5β-dihydrotestosterone), 3α,5β (etiocholanolone); BP (blood pressure), Tfm (testiscular-feminized male), SHR (spontaneously hypertensive rats), ORX (orchidectomized), WKY (Wistar Kyoto).

It seems that physiological androgen levels regulate BP by placing a "brake" for developing hypertension, and indeed hypotestosteronemia is a risk factor for hypertension. Several studies have reported that men with CVD, type 2 diabetes mellitus, obesity, metabolic syndrome and dyslipidemia have low levels of TES (reviewed in [46,53,71,87,88]). In this respect, the incidence of CVD increases in aging men when TES production decreases. In line with these findings, it has been suggested that hypertension, a comorbidity highly susceptible to increased COVID-19 severity, may be due to lower androgen levels in older men patients [89]. These findings indicate that TES is beneficial for the cardiovascular system.

In parallel, these findings have also shown that DHEA and 5β-DHT are significantly more potent than TES in evoking an antihypertensive response, indicating that androgens may provide a therapeutic potential for the regulation of BP.

### 2.3. Mechanism of vasorelaxing action of androgens

#### 2.3.1. Non-genomic action

The action of androgens in inducing vasorelaxation has been defined as follows: androgen–induced vasorelaxation is rapid (~2 min). Likewise, several lines of pharmacological evidence have established that this immediate effect induced by androgens is non-genomically mediated, since the vasorelaxing effect of androgens is observed in the presence of AR antagonists, protein synthesis, and transcription inhibitors [51,52,90,91]. The response is observed even when the androgen is conjugated to molecules, such as bovine serum albumin (BSA), which prevents it from entering the cytoplasm [92], and the AR deficient testicular-feminized rat inhibitors [90]. Indeed, these data taken together indicate that the mechanism of action of androgens is unlikely to involve nuclear receptor activation.

#### 2.3.2. Endothelium- and nitric oxide (NO)-independent mechanism

Numerous studies have reported that the vasorelaxing effect caused by 17β-estradiol is mediated in part by via generation of endothelium-derived NO and is attenuated by NO synthesis inhibitors, suggesting an endothelium-dependent mechanism [37], revised in [93] but this seems not to be the case for androgens. Androgen-induced vasorelaxation is observed in endothelium-denuded vascular preparations, as well as in the presence of NO synthase inhibitors such as L-NAME [43,51,52,94–97], as revised by [20]. These findings indicate that androgen-induced vasorelaxation is endothelium–independent. In this

respect, it is relevant to consider the following convincing facts: (i) androgens are powerful vasorelaxants in the human umbilical artery [51], notably, this blood vessel has an atypical endothelium due to the fact that biosynthesis of endothelial NO is absent; and (ii) androgen-induced antihypertensive responses in preeclamptic rats [83]. Clinical and experimental studies have suggested that endothelial dysfunction is present in preeclamptic subjects [98–102]. In summary, the vasorelaxing effect of androgens strongly suggests an NO–independent mechanism [51].

In a marked contrast, some contradictory data have reported that androgen-induced vasorelaxation is **partially**, but not completely, inhibited through pretreatment with L-NAME [103–106], suggesting that a partial endothelium-dependent mechanism might be involved. A study also documented that vasodilatation to TES in human pulmonary arteries is dependent upon the presence of an intact endothelium [57]. However, this contradiction may be related to differences in the vessel type or experimental animal species and/or, as previously suggested by Jones and Kelly [88], can also be explained by the fact that TES is converted to 17β-estradiol (see Fig. 1); an estrogen that mediates its vasorelaxing effect through stimulation of NO production, it is currently not know whether the reported vasorelaxing effect of TES is a direct endothelium-dependent effect or an indirect effect via estrogens.

Although these data suggest that the endothelium is not involved in androgen-induced vasorelaxation, further investigations are needed to clarify this controversy.

### 2.3.3. Ion channels

We provided the first experimental evidence that the mechanism of vasorelaxation of androgens was due to the blockage of voltage- and receptor-dependent $Ca^{2+}$ channels, a mechanism that restricts the availability of extracellular $Ca^{2+}$ in the contractile machinery [94,95]. Vasorelaxation of 17β-estradiol, progesterone, and TES, inhibited the entry of $Ca^{2+}$ but not its release [107]. More specifically, in single vascular myocytes, it has been shown that the mechanism of action of androgens in inducing vasorelaxation depends on the blockade of L–voltage-operated $Ca^{2+}$ channels (L-VOCCs) [91,97,108–110], revised by [88]. A compelling fact is that TES acts on the same site as dihydropyridines (nifedipine), the $\alpha_{1C}$ subunit of the L-VOCCs, in order to block the extracellular $Ca^{2+}$ entry into intracellular space [110]. We also found that 5β-DHT acts exclusively as an antagonist to blocking $Ca^{2+}$ currents via L-VOCCs (from nM to μM concentrations), whereas TES possesses different mechanisms: (i) a dual effect on L-VOCCs (antagonist at nM concentrations/agonist at μM concentrations), (ii) an increase in $[Ca^{2+}]_i$ and (iii) an increase in cAMP production. This is the first evidence which can help us understand the mechanism underlying the acute vasorelaxing action of 5β-DHT and the controversial response of TES upon vascular reactivity, i.e., the vasorelaxing and/or anti-vasorelaxing effects [91].

Several functional studies in VSM and other SMs demonstrated the TES may also relax contractions induced by KCl and several contractile agonists, such as: acetylcholine, carbachol, noradrenaline, oxytocin, ovalbumin, phenylephrine, prostaglandins, and serotonin, among others, it cannot be rule out that androgens may also inactive non-voltage-gated pathways, i.e. the store operated $Ca^{2+}$ entry (SOCE) and receptor-operated $Ca^{2+}$ channels (ROCCs), suggesting that TES has an antagonistic effect on L-VOCCs, ROCCs, and/or SOCE, showing that the vasorelaxation is induced by blockade of extracellular $Ca^{2+}$ entry through these channels. In this respect, in addition there is also the experimental findings that in the bronchodilating effect of androgens in airway SM [35] SOCE and ROCC are partly involved.

In addition to the above, a human study examined the effect of TES therapy on vascular reactivity in isolated human subcutaneous arteries it was observed that after TES therapy, the vasoconstrictor effect of noradrenaline increased and reduced the vasodilator response to acetylcholine and sodium nitroprusside (which breaks down to NO in the circulation). This effect physiologically would divert blood flow toward the vital organs, an important survival effect in chronic heart failure [85], revised in [88] which is an evidence for a different mode of action of test on vascular reactivity.

In addition to $Ca^{2+}$ channels, it has been repeatedly documented that functional studies in some vascular beds, TES-induced vasorelaxation may also activate several types of $K^+$ channels, which are: voltage sensitive $K^+$ channels ($K_V$), large-conductance $Ca^{2+}$-activated $K^+$ channels ($BK_{Ca}$), ATP-sensitive $K^+$ channels ($K_{ATP}$), and inward-rectifier $K^+$ channels ($K_{IR}$) [56,92,96,103,111]. One study through patch-clamp experiments reported that TES produced activation of $BK_{Ca}$ channels [96]. In conclusion, the main $K^+$ channels involved in this process are $BK_{Ca}$ and $K_v$.

With these findings, there is controversy. The most intriguing conclusions, which can be drawn from the numerous observations of non-genomic vasorelaxing mechanisms are as follows: either (i) the mechanism differs depending blood vessels [52]; or (ii) in agreement with [21], androgen-induced vasorelaxation is due to the fact that $Ca^{2+}$ channels are closely related to $K^+$ channels, where membrane hyperpolarization by $K^+$ channel activation closes the $Ca^{2+}$ channels and leads to vasorelaxation.

However, little attention has been paid to the *in vivo* mechanism of action. The mechanism of androgen action in eliciting antihypertensive responses was studied for the first time in conscious (*in vivo*) rats. 5β-DHT abolished the pressor response to Bay K 8644 (an L-VOCC antagonist) *in vivo*, suggesting a blockade of L-VOCC [82]. Our data in conscious orchidectomized hypertensive rats revealed that: (i) the blockade of NO synthase by L-NAME did not alter the antihypertensive response to 5β-DHT; and (ii) endothelin-1, by binding to $ET_A$ receptors, interferes with NO synthesis and acts to counter NO-induced vasodilation; under these circumstances, dose-dependent antihypertension induced by 5β-DHT was not abolished in the presence of L-NAME or endothelin-1 [84].

Fig. 3 illustrates the mechanisms of action of androgens described here to produce vasorelaxation/hypotension.

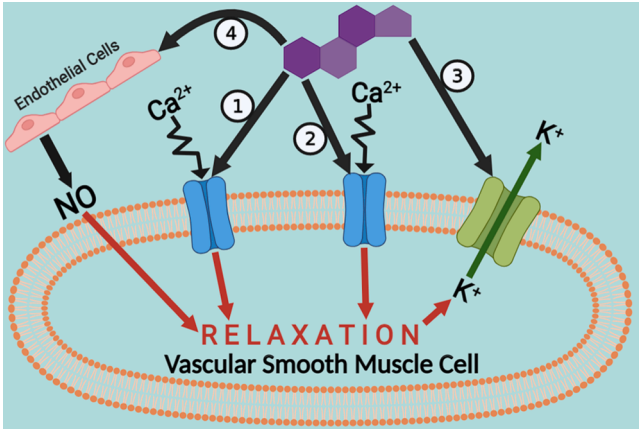



**Fig. 3. The most recognized non-genomic mechanisms of vasorelaxing/hypotensive action.** 1) Restriction of extracellular $Ca^{2+}$ entry by androgens to block L-type voltage-operated $Ca^{2+}$ channel (L-VOCCs). TES shares the same molecular target as the dihydropyridines (the $\alpha_{1C}$ subunit of L-VOCCs). 2) Restriction of extracellular $Ca^{2+}$ entry through blockage of the receptor–operated $Ca^{2+}$ channels (ROCCs). Restricting extracellular $Ca^{2+}$ entry and diminishing intracellular $Ca^{2+}$ concentration in the vascular smooth muscle cell to induce vasorelaxation and hypotensive response. 3) TES activates voltage sensitive $K^+$ channels ($K_v$) or large-conductance–$Ca^{2+}$-activate $K^+$ channels ($K_{Ca}$), increasing $K^+$ efflux to induce VSM hyperpolarization and vasorelaxation. 4) A partial endothelium-dependent mechanism has been suggested, presumably involving endothelium-derived relaxing factors from endothelial cells, particularly nitric oxide (NO), would seem to be responsible for the vasorelaxing effect of TES; however, a controversy exists (see discussion in 2.3 section).

## 3. Differences between male and female steroid hormones in cardiovascular system

It is relevant to note that when the efficacies and potencies of androgen-induced vasorelaxation were compared to female sex steroids, it was observed that TES and its two immediate dihydrometabolites, 5α- and 5β-DHT, but not TES, have turned out to be more potent vasorelaxants than the female steroids, estrogens and progestins in inducing vasorelaxation (see section 5). Our early observations were that 5β-DHT was the most potent steroid, even when compared to the vasorelaxing response of progestins in the rat aorta [94]. Remarkably, the concentration–response curve to 5β-DHT exhibited the highest potency for vasorelaxation of the human umbilical artery, even exceeding the potency of several progestins [51]. This is probably the only published report comparing the efficacy and potency of TES and its 5-reduced metabolites with progesterone and its 5-reduced progestins.

Regarding estrogens, the concentration–response curves showed that 5β-DHT was significantly more potent than 17β-estradiol in inducing vasorelaxation in rat aorta [95]. In the pulmonary vasculature, the vasorelaxing efficacy of 17β-estradiol was significantly lower than that of TES [47]. In patch-clamp electrophysiological studies, the concentration–response curves to TES, 5β-DHT and 17β-estradiol on $Ca^{2+}$ currents in single aortic myocytes from rats indicated that androgens were more potent in blocking $Ca^{2+}$ currents than 17β-estradiol [91].

Despite these findings, studies by Khalil and colleagues demonstrated that progesterone and TES are less potent than 17β-estradiol in inducing vasorelaxation [107,112,113]. In this respect, it is important to note that TES is a less potent vasorelaxant than DHEA and its 5β-reduced metabolite; thus, the roles of TES have been inappropriately generalized as common to all androgens (see section 5).

However, in a general context, androgens, particularly 5β-DHT, have turned out to be more potent vasorelaxants than the female steroids, estrogens, and progestins.

## 4. Sex differences in vasorelaxing effects of androgens

Historically, female sex steroid hormones (estrogens and progestins) have been studied extensively, which has led to substantial understanding of human female physiology and the diverse effects of these hormones on physiological, as well as reproductive functions.

A huge body of evidence has created the wrong belief that estrogens are cardioprotectors in females and that androgens are detrimental to the male cardiovascular system. Recently, the interest in the role of sex regulation of metabolic homeostasis, BP, and CVD has been growing. Men typically develop CVD earlier than women [66]; however, it is important to consider that very few studies have examined androgen signaling and hypertension in females.

Some studies have suggested that androgens are as important for BP regulation in females as they are in males. The available data suggest that *in vitro* relaxation induced by TES is independent of sex. No significant differences between the vasorelaxing effect of TES in the coronary arteries and aortas of male *vs* female rabbits were observed [90], or in the aortas of male *vs* female rats [114]. TES has also been reported to induce sex-independent vasodilation *in vivo* in canine coronary conductance and resistance arteries [104]. TES elicits vasorelaxation in both males and females, however, rat pulmonary and coronary arteries in males were more sensitive to the effects of TES than those in female [47].

In addition, androgens also possess the ability to relax the female human vasculature, such as the internal mammary artery [55], pulmonary arteries and veins [57] and umbilical arteries [51]. This effect of TES on the human umbilical artery has also been confirmed by others [56]. Moreover, recently it has been reported that androgens produce antihypertensive responses in an *in vivo* preeclampsia rat model [83].

Therefore, these studies have concluded that androgen-induced relaxation of VSM appears to be sex-independent, which indicates that male sex hormones may also regulate physiological processes in both women and men.

## 5. Molecular changes in androgen structure associated to vascular reactivity

Relatively few studies have documented the effects of other androgens, metabolites, and analogs of TES on VSM, which is of considerable interest. The 5-reduced non-aromatized metabolites of TES (5α-, 5β-DHT, and a few terahydo metabolites) also induce vasorelaxation, demonstrating that these steroid metabolites are also vasoactive steroids.

Our research group has systematically studied the vascular effects of androgen metabolites and reported that the vasorelaxing effects of 5β-DHT are notably more efficacious and potent than those of its precursor, TES, in the rat aorta [20,86,91,94,95], dog coronary and femoral arteries [52], pig coronary and prostatic small arteries [96,115], and in the human umbilical artery [51]. Taken together, these findings provide clear evidence that 5β-DHT exhibits the highest efficacy and potency in inducing vasorelaxation in isolated blood vessels, even when compared to the vasorelaxing effects of progestins and estrogens [47,91,94,95].

In a marked contrast, the 5α isomer (5α-DHT) of 5β-DHT, was found to be less potent or equipontent to TES in relaxing of the rat aorta [92], dog coronary and femoral arteries [52], pig coronary and prostatic small arteries [96,115], and human umbilical arteries [51,51]. Similar studies have shown that TES and 5α-DHT may also relax human penile arteries [58,59], cavernosal arteries [60] and internal spermatic veins [61] without any differences in their relaxing efficacy or potency. Moreover, as a nonaromatizable dihydro-androgen metabolite of TES, 5α–DHT, has been frequently used to verify that aromatization of TES into estrogen is not required for this androgen to produce vasorelaxation [92,96,103,104].

The subsequent 3α or 3β hydroxylation of the 5α- and 5β-reduced dihydro metabolites are generally less potent than TES in inducing vasorelaxation [61], although androsterone was found to be equipotent to TES [51,92].

On the other hand, the esters of TES, which, by virtue of their greater lipid solubility, exhibit longer acting androgenic effects *in vivo,* are less efficacious and potent than TES in inducing vasorelaxation *in vitro*. Thus, TES-enanthate and TES-hemisuccinate were less efficacious and potent than TES in the rat aorta [92] and in rabbit coronary arteries and aortas [90]. In isolated human vessels, TES-propionate was found to relax the internal spermatic vein [61] and to be more efficacious than TES-phenylpropionate, TES-decanoate, and TES-isocaproate in relaxing the radial and internal mammary arteries and spermatic veins [55,111]. However, the weakness of these studies was their failure to include TES and/or its metabolites as a control group.

Consistent with the *in vitro* effects of TES metabolites on vasorelaxation, studies have shown that the 5-reduced metabolites of TES are also capable of blocking vasopressor responses of noradrenaline or Bay K 8644 in vagosympathectomized, pithed rats *in vivo*. Again, 5β-DHT was more potent than 5α-DHT or the 3α,5β-reduced metabolite in inducing a rapid vasodepressor response [80]. Similarly, TES and 5β-DHT both produced dose-dependent reductions in the mean arterial BP of conscious male normotensive and testicular-feminized male rats (Tfm), and 5β–DHT was found to produce greater reductions in BP than TES [81]. Likewise, the order of potency of producing an acute antihypertensive response in conscious male spontaneously hypertensive rats (SHR) was as follows: 5β-DHT > TES > 5α-DHT [82].

Conformational analysis of different androgens showed that there is a difference between the 5α- and 5β-reduced metabolites. Ring A in the steroid molecule of 5β-steroids is folded under or below the plane of the remainder of the molecule, similarly to Δ4-3keto configuration observed in TES, Δ5,3β hydroxy structure in DHEA, and the 5α-reduction (5α-DHT) with an A/B *trans* ring–junction (see molecular structures in Fig. 1). Thus, the A/B *cis* conformation in 5β-DHT is responsible of a

major non-genomic efficacy and potency to induce vasorelaxation and subsequently reduction of BP than that induced by TES and 5α-DHT.

Remarkably, 5β-reduction does not bind to the AR and is completely devoid of androgenic responses. This androgen is also non-aromatizable and is deprived of estrogenic properties; nonetheless, possesses acute vasorelaxing and antihypertensive responses non–genomically mediated. Therefore, 5β-DHT is not a biologically inactive metabolite. 5β–Reduced steroids are increasingly being recognized as active compounds with important regulatory functions. The present review highlights the role that this 5β-reduced androgen plays in the regulation of BP and that it is an excellent therapeutic option. The cited findings regarding the vasorelaxing, hypotensive and the antihypertensive responses indicate that the beneficial actions of androgens might be very relevant for understanding and treating hypertension, which is one of the most important health problems worldwide. In support of this view, there is direct evidence indicating that the activity of 5β-reductase, the enzyme that catalyzes the conversion of TES into 5β-DHT, is significantly lower in patients with essential hypertension than in normotensive controls [116].

Furthermore, it should be noted that although castration-induced prostate regression is a well-known phenomenon, this is not a good alternative for treating prostatic cancer. Our findings suggest that the reduction in prostatic blood flow may be treated by using 5β-DHT, which is devoid of androgenic and anabolic properties due to its lack of genomic activity; however, this androgen is capable of regulating prostatic function, similarly to TES, through control of blood flow accompanied by its vasorelaxing and antihypertensive non-genomic responses.

Finally, of considerable interest is to note a frequent mistake in scientific literature: the 5–reduced dihydroandrogens (5α- and 5β-DHT) are only identified as "DHT," and relatively few studies distinguish between the 5α or 5β configurations; however, it has become clear that the biological effects of these molecular configurations are fundamentally different.

Admittedly, the type and dose of androgen, as well as the future route of administration of androgen replacement therapies, will require substantially more investigation.

## 6. Perspectives

In an optimistic manner, the present lines of evidence can conclude that C19-steroids (androgens) in general, and 5β-DHT in particular, are vasoactive male sex steroids, which may play an important role in cardiovascular regulation. A deficiency in endogenous TES may be a risk factor for hypertension and CVD, and exogenous androgens may be used therapeutically for the prevention or treatment of hypertension. Although a deficiency in either TES or 5α-DHT may be involved in the development of hypertension, treatment with 5β-DHT would be preferred, since it not only exhibits a higher efficacy and potency than TES, but also lacks genomic effects on reproductive target tissues. In summary, the properties of 5β-DHT i.e., no bioconversion to estrogens lacking estrogenic effects and androgenic action, and outstanding vasorelaxing action, point to this androgen as an excellent candidate for hypertension control. Taking into account, the rapid non-genomic effect (1–2 min) of androgens in inducing vasorelaxation and consequently reduction of BP, particularly 5β-DHT might be administered intravenously as a bolus and could be considered as a first-line therapy for acute hypertensive emergency. Admittedly, further clinical studies are urgently needed.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

I can share data in the present Review

*Acknowledgement*

The author would like to thank Nieves Herrera for organizing the references in this manuscript.

Part of this work was supported by a grant from *Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica-UNAM* (PAPIIT), grant number IN200320 to M. Perusquía.

## References

[1] A.D. Mooradian, J.E. Morley, S.G. Korenman, Biological actions of androgens, Endocr. Rev. 8 (1987) 1–28.
[2] K. Rana, R.A. Davey, J.D. Zajac, Human androgen deficiency: insights gained from androgen receptor knockout mouse models, Asian, J. Androl. 16 (2014) 169–177.
[3] L. Schiffer, W. Arlt, K.H. Storbecka, Intracrine androgen biosynthesis, metabolism and action revisited, Mol. Cell. Endocrinol. 465 (2018) 4–26.
[4] R.J. Young, A.H. Ismail, A. Bradley, D.L. Corrigan, Effect of prolonged exercise of serum testosterone levels in adult men, Br. J. Sports Med. 10 (1976) 230–235.
[5] J.R. Givens, R.N. Andersen, W.L. Wiser, A.J. Donelson, S.A. Coleman, A testosterone-secreting, gonadotropin-responsive pure thecoma and polycystic ovarian disease, J. Clin. Endocrinol. Metab. 41 (1975) 845–853.
[6] E. Pretorius, D.J. Africander, M. Vlok, M.S. Perkins, J. Quanson, K.H. Storbeck, 11-Ketotestosterone and 11-Ketodihydrotestosterone in Castration Resistant Prostate Cancer: Potent Androgens Which Can No Longer Be Ignored, PLoS One 11 (2016), e0159867.
[7] J. Rege, Y. Nakamura, F. Satoh, R. Morimoto, M.R. Kennedy, L.C. Layman, et al., Liquid chromatography-tandem mass spectrometry analysis of human adrenal vein 19-carbon steroids before and after ACTH stimulation, J. Clin. Endocrinol. Metab. 98 (2013) 1182–1188.
[8] K.H. Storbeck, L.M. Bloem, D. Africander, L. Schloms, P. Swart, A.C. Swart, 11beta-Hydroxydihydrotestosterone and 11-ketodihydrotestosterone, novel C19 steroids with androgenic activity: a putative role in castration resistant prostate cancer? Mol. Cell. Endocrinol. 377 (2013) 135–146.
[9] E.M. Wilson, F.S. French, Binding properties of androgen receptors, Evidence for identical receptors in rat testis, epididymis, and prostate, J. Biol. Chem. 251 (1976) 5620–5629.
[10] A.M. Matsumoto, Testosterone administration in older men, Endocrinol. Metab. Clin. North. Am. 42 (2013) 271–286.
[11] P.B. Grino, J.E. Griffin, J.D. Wilson, Testosterone at high concentrations interacts with the human androgen receptor similarly to dihydrotestosterone, Endocrinology 126 (1990) 1165–1172.
[12] C.A. Heinlein, C. Chang, Androgen receptor (AR) coregulators: an overview, Endocr. Rev. 23 (2002) 175–200.
[13] C.A. Quigley, B.A. De, K.B. Marschke, M.K. el-Awady, E.M. Wilson, F.S. French, Androgen receptor defects: historical, clinical, and molecular perspectives, Endocr. Rev. 16 (1995) 271–321.
[14] R. Losel, M. Wehling, Nongenomic actions of steroid hormones, Nat. Rev. Mol. Cell. Biol. 4 (2003) 46–56.
[15] P. Babica, R. Zurabian, E.R. Kumar, R. Chopra, M.J. Mianecki, J.S. Park, et al., Methoxychlor and Vinclozolin Induce Rapid Changes in Intercellular and Intracellular Signaling in Liver Progenitor Cells, Toxicol. Sci. 153 (2016) 174–185.
[16] M. Wehling, Specific, nongenomic actions of steroid hormones, Annu. Rev. Physiol. 59 (1997) 365–393.
[17] R.M. Lösel, E. Falkenstein, M. Feuring, A. Schultz, H.C. Tillmann, K. Rossol-Haseroth, et al., Nongenomic steroid action: controversies, questions, and answers, Physiol. Rev. 83 (2003) 965–1016.
[18] M. Perusquía, Androgen-induced vasorelaxation: a potential vascular protective effect, Exp. Clin. Endocrinol. Diabetes 111 (2003) 55–59.
[19] C.D. Foradori, M.J. Weiser, R.J. Handa, Non-genomic actions of androgens, Front. Neuroendocrinol. 29 (2008) 169–181.
[20] M. Perusquía, J.N. Stallone, Do androgens play a beneficial role in the regulation of vascular tone? Nongenomic vascular effects of testosterone metabolites, Am. J. Physiol. Heart. Circ. Physiol. 298 (2010) H1301–H1307.
[21] M. Lorigo, M. Mariana, M.C. Lemos, E. Cairrao, Vascular mechanisms of testosterone: The non-genomic point of view, J. Steroid Biochem. Mol. Biol. 196 (2020), 105496.
[22] I.G. Brodsky, P. Balagopal, K.S. Nair, Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men-a clinical research center study, J. Clin. Endocrinol. Metab. 81 (1996) 3469–3475.
[23] R.J. Urban, Y.H. Bodenburg, C. Gilkison, J. Foxworth, A.R. Coggan, R.R. Wolfe, et al., Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis, Am. J. Physiol. 269 (1995) E820–E826.
[24] A.T. Kicman, Pharmacology of anabolic steroids, Br. J. Pharmacol. 154 (2008) 502–521.
[25] M. Perusquía, Nongenomic action of steroids in myometrial contractility, Endocrine 15 (2001) 63–72.

[26] M. Perusquía, E. Navarrete, J. Jasso-Kamel, L.M. Montaño, Androgens induce relaxation of contractile activity in pregnant human myometrium at term: a nongenomic action on L-type calcium channels, Biol. Reprod. 73 (2005) 214–221.
[27] S. Makieva, P.T. Saunders, J.E. Norman, Androgens in pregnancy: roles in parturition, Hum. Reprod. Update. 20 (2014) 542–559.
[28] C. Kubli-Garfias, C. Hoyo-Vadillo, H. Ponce-Monter, Relaxant effect of testosterone and 5 alpha-reduced androgens on the smooth muscle of the male rat reproductive system, Proc. West. Pharmacol. Soc. 26 (1983) 31–34.
[29] O. Yildiz, M. Seyrek, H.C. Irkilata, I. Yildirim, L. Tahmaz, M. Dayanc, Testosterone might cause relaxation of human corpus cavernosum by potassium channel opening action, Urology 74 (2009) 229–232.
[30] V.S. Fernandes, M.V. Barahona, P. Recio, A. Martinez-Saenz, A.S. Ribeiro, C. Contreras, Mechanisms involved in testosterone-induced relaxation to the pig urinary bladder neck, Steroids 77 (2012) 394–402.
[31] K.L. Hristov, S.P. Parajuli, A. Provence, G.V. Petkov, Testosterone decreases urinary bladder smooth muscle excitability via novel signaling mechanism involving direct activation of the BK channels, Am. J. Physiol. Renal. Physiol. 311 (2016) F1253–F1259.
[32] Y. Ishida, H. Oshima, S. Aibara, M. Ohmoto, Inhibitory actions of steroid hormones on isolated smooth muscles, Yakugaku Zasshi. 92 (1972) 1175–1179.
[33] C. Kubli-Garfias, M. Medina-Jimenez, E. Garcia-Yanez, A. Vazquez-Alvarez, M. Perusquía, N. Gomez-Garcia, Relaxant action of androgens, progestins and corticosteroids on the isolated ileum of the guinea pig, Acta Physiol. Pharmacol. Latinoam. 37 (1987) 357–364.
[34] L.W. Kline, E. Karpinski, Testosterone and dihydrotestosterone inhibit gallbladder motility through multiple signalling pathways, Steroids 73 (2008) 1174–1180.
[35] L.M. Montaño, E. Flores-Soto, B. Sommer, H. Solís-Chagoyán, M. Perusquía, Androgens are effective bronchodilators with anti-inflammatory properties: A potential alternative for asthma therapy, Steroids 153 (2020), 108509.
[36] M.E. Mendelsohn, R.H. Karas, The protective effects of estrogen on the cardiovascular system, N. Engl. J. Med. 340 (1999) 1801–1811.
[37] R.K. Dubey, E.K. Jackson, Estrogen-induced cardiorenal protection: potential cellular, biochemical, and molecular mechanisms, Am. J. Physiol. Renal. Physiol. 280 (2001) F365–F388.
[38] P. Alexandersen, J. Haarbo, I. Byrjalsen, H. Lawaetz, C. Christiansen, Natural androgens inhibit male atherosclerosis: a study in castrated, cholesterol-fed rabbits, Circ. Res. 84 (1999) 813–819.
[39] P.J. Pugh, R.D. Jones, J.N. West, T.H. Jones, K.S. Channer, Testosterone treatment for men with chronic heart failure, Heart 90 (2004) 446–447.
[40] C.J. Malkin, P.J. Pugh, J.N. West, E.J. Van Beek, T.H. Jones, K.S. Channer, Testosterone therapy in men with moderate severity heart failure: a double-blind randomized placebo controlled trial, Eur. Heart J. 27 (2006) 57–64.
[41] T.H. Jones, Testosterone deficiency: a risk factor for cardiovascular disease? Trends. Endocrinol. Metab. 21 (2010) 496–503.
[42] G.M. Rosano, F. Leonardo, P. Pagnotta, F. Pelliccia, G. Panina, E. Cerquetani, et al., Acute anti-ischemic effect of testosterone in men with coronary artery disease, Circulation 99 (1999) 1666–1670.
[43] C.M. Webb, J.G. McNeill, C.S. Hayward, D. de Zeigler, P. Collins, Effects of testosterone on coronary vasomotor regulation in men with coronary heart disease, Circulation 100 (1999) 1690–1696.
[44] W. Pongkan, S.C. Chattipakorn, N. Chattipakorn, Roles of Testosterone Replacement in Cardiac Ischemia-Reperfusion Injury, J. Cardiovasc. Pharmacol. Ther. 21 (2016) 27–43.
[45] G.B. Phillips, T.Y. Jing, L.M. Resnick, M. Barbagallo, J.H. Laragh, J.E. Sealey, Sex hormones and hemostatic risk factors for coronary heart disease in men with hypertension, J. Hypertens. 11 (1993) 699–702.
[46] K.M. English, O. Mandour, R.P. Steeds, M.J. Diver, T.H. Jones, K.S. Channer, Men with coronary artery disease have lower levels of androgens than men with normal coronary angiograms, Eur. Heart J. 21 (2000) 890–894.
[47] K.M. English, R.D. Jones, T.H. Jones, A.H. Morice, K.S. Channer, Gender differences in the vasomotor effects of different steroid hormones in rat pulmonary and coronary arteries, Horm. Metab. Res. 33 (2001) 645–652.
[48] A.M. Traish, A. Guay, R.J. Feeley, F. Saad, The dark side of testosterone deficiency: I. Metabolic syndrome and erectile dysfunction, J. Androl. 30 (2009) 10–22.
[49] A.B. Araujo, J.M. Dixon, E.A. Suarez, M.H. Murad, L.T. Guey, G.A. Wittert, Clinical review: Endogenous testosterone and mortality in men: a systematic review and meta-analysis, J. Clin. Endocrinol. Metab. 96 (2011) 3007–3019.
[50] G. Corona, E. Maseroli, G. Rastrelli, A.M. Isidori, A. Sforza, E. Mannucci, et al., Cardiovascular risk associated with testosterone-boosting medications: a systematic review and meta-analysis, Expert. Opin. Drug. Saf. 13 (2014) 1327–1351.
[51] M. Perusquía, E. Navarrete, L. Gonzalez, C.M. Villalon, The modulatory role of androgens and progestins in the induction of vasorelaxation in human umbilical artery, Life Sci. 81 (2007) 993–1002.
[52] M. Perusquía, J. Espinoza, L.M. Montaño, J.N. Stallone, Regional differences in the vasorelaxing effects of testosterone and its 5-reduced metabolites in the canine vasculature, Vascul. Pharmacol. 56 (2012) 176–182.
[53] D.M. Kelly, T.H. Jones, Testosterone: a vascular hormone in health and disease, J. Endocrinol. 217 (2013) R47–R71.
[54] M. Lorigo, M. Mariana, N. Oliveira, M.C. Lemos, E. Cairrao, Vascular Pathways of Testosterone: Clinical Implications, J. Cardiovasc. Transl. Res. 13 (2020) 55–72.
[55] O. Yildiz, M. Seyrek, H. Gul, I. Un, V. Yildirim, E. Ozal, et al., Testosterone relaxes human internal mammary artery in vitro, J. Cardiovasc. Pharmacol. 45 (2005) 580–585.
[56] E. Cairrão, E. Alvarez, A.J. Santos-Silva, I. Verde, Potassium channels are involved in testosterone-induced vasorelaxation of human umbilical artery, Naunyn Schmiedebergs Arch. Pharmacol. 376 (2008) 375–383.
[57] K.O. Rowell, J. Hall, P.J. Pugh, T.H. Jones, K.S. Channer, R.D. Jones, Testosterone acts as an efficacious vasodilator in isolated human pulmonary arteries and veins: evidence for a biphasic effect at physiological and supra-physiological concentrations, J. Endocrinol. Invest. 32 (2009) 718–723.
[58] K. Nagao, T. Tai, R. Saigo, M. Kimura, N. Tanaka, H. Kobayashi, et al., Role of androgen in the elderly. Male sexual dysfunction and androgen, Clin. Calcium 23 (2013) 1163–1169.
[59] X.H. Zhang, A. Melman, M.E. Disanto, Update on corpus cavernosum smooth muscle contractile pathways in erectile function: a role for testosterone? J. Sex. Med. 8 (2011) 1865–1879.
[60] E. Waldkirch, S. Uckert, D. Schultheiss, U. Geismar, C. Bruns, F. Scheller, et al., Non-genomic effects of androgens on isolated human vascular and nonvascular penile erectile tissue, BJU Int. 101 (2008) 71–75.
[61] M. Seyrek, H.C. Irkilata, I.M. Vural, I. Yildirim, S. Basal, O. Yildiz, et al., Testosterone relaxes human internal spermatic vein through potassium channel opening action, Urology 78 (2011) 233e1-5.
[62] Y. Shibata, B. Kashiwagi, S. Arai, Y. Fukabori, K. Suzuki, S. Honma, et al., Direct regulation of prostate blood flow by vascular endothelial growth factor and its participation in the androgenic regulation of prostate blood flow in vivo, Endocrinology 145 (2004) 4507–4512.
[63] A.M. Traish, I. Goldstein, N.N. Kim, Testosterone and erectile function: from basic research to a new clinical paradigm for managing men with androgen insufficiency and erectile dysfunction, Eur. Urol. 52 (2007) 54–70.
[64] K.M. English, R.P. Steeds, T.H. Jones, M.J. Diver, K.S. Channer, Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina: A randomized, double-blind, placebo-controlled study, Circulation 102 (2000) 1906–1911.
[65] A. Mathur, C. Malkin, B. Saeed, R. Muthusamy, T.H. Jones, K. Channer, Long-term benefits of testosterone replacement therapy on angina threshold and atheroma in men, Eur. J. Endocrinol. 161 (2009) 443–449.
[66] R. Maranon, J.F. Reckelhoff, Sex and gender differences in control of blood pressure, Clin. Sci. (Lond) 125 (2013) 311–318.
[67] H. Selye, Anesthetic Effect of Steroid Hormones, Soc. Exp. Biol. Med. 46 (1941) 116–121.
[68] H. Selye, Correlations between the chemical structure and the pharmacological actions of the steroids, Endocrinology 30 (1942) 437–453.
[69] K.T. Khaw, E. Barrett-Connor, Blood pressure and endogenous testosterone in men: an inverse relationship, J. Hypertens. 6 (1988) 329–332.
[70] G.B. Phillips, B.H. Pinkernell, T.Y. Jing, The association of hypotestosteronemia with coronary artery disease in men, Arterioscler. Thromb. 14 (1994) 701–706.
[71] K.M. English, R. Steeds, T.H. Jones, K.S. Channer, Testosterone and coronary heart disease: is there a link? QJM 90 (1997) 787–791.
[72] P.J. Pugh, K.M. English, T.H. Jones, K.S. Channer, Testosterone: a natural tonic for the failing heart? QJM 93 (2000) 689–694.
[73] J. Svartberg, M.D. von, H. Schirmer, E. Barrett-Connor, J. Sundfjord, R. Jorde, Association of endogenous testosterone with blood pressure and left ventricular mass in men. The Tromso Study, Eur. J. Endocrinol. 150 (2004) 65–71.
[74] R. Haring, U. John, H. Volzke, M. Nauck, M. Dorr, S.B. Felix, et al., Low testosterone concentrations in men contribute to the gender gap in cardiovascular morbidity and mortality, Gend. Med. 9 (2012) 557–568.
[75] P. Pietri, C. Vlachopoulos, N. Ioakeimidis, A. Aggelis, D. Terentes-Printzios, E. Paschalidis, et al., Association of plasma testosterone with central haemodynamics in hypertensive men, J. Hypertens. 33 (2015), e67.
[76] Y. Jiang, J. Ye, M. Zhao, A. Tan, H. Zhang, Y. Gao, et al., Cross-sectional and longitudinal associations between serum testosterone concentrations and hypertension: Results from the Fangchenggang Area Male Health and Examination Survey in China, Clin. Chim. Acta 487 (2018) 90–95.
[77] P.J. Pugh, T.H. Jones, K.S. Channer, Acute haemodynamic effects of testosterone in men with chronic heart failure, Eur. Heart J. 24 (2003) 909–915.
[78] A. Haider, A. Yassin, K.S. Haider, G. Doros, F. Saad, G.M. Rosano, Men with testosterone deficiency and a history of cardiovascular diseases benefit from long-term testosterone therapy: observational, real-life data from a registry study, Vasc. Health Risk Manag. 12 (2016) 251–261.
[79] Q. Yang, Z. Li, W. Li, L. Lu, H. Wu, Y. Zhuang, Association of total testosterone, free testosterone, bioavailable testosterone, sex hormone-binding globulin, and hypertension, Medicine (Baltimore) 98 (2016), e15628.
[80] M. Perusquía, C.M. Villalón, The vasodepressor effect of androgens in pithed rats: potential role of calcium channels, Steroids 67 (2002) 1021–1028.
[81] M. Perusquía, C.D. Greenway, L.M. Perkins, J.N. Stallone, Systemic hypotensive effects of testosterone are androgen structure-specific and neuronal nitric oxide synthase-dependent, Am. J. Physiol. Regul. Integr. Comp. Physiol. 309 (2015) R189–R195.
[82] M. Perusquía, N. Herrera, M. Ferrer, J.N. Stallone, Antihypertensive effects of androgens in conscious, spontaneously hypertensive rats, J. Steroid Biochem. Mol. Biol. 167 (2017) 106–114.
[83] M. Perusquía, A.E. Hanson, C.M. Meza, C. Kubli, N. Herrera, J.N. Stallone, Antihypertensive responses of vasoactive androgens in an in vivo experimental model of preeclampsia, J. Steroid Biochem. Mol. Biol. 178 (2018) 65–72.
[84] M. Perusquía, D. Contreras, N. Herrera, Hypotestosteronemia is an important factor for the development of hypertension: elevated blood pressure in orchidectomized conscious rats is reversed by different androgens, Endocrine 65 (2019) 416–425.

[85] C.J. Malkin, R.D. Jones, T.H. Jones, K.S. Channer, Effect of testosterone on ex vivo vascular reactivity in man, Clin. Sci. (Lond) 111 (2006) 265–274.
[86] L. Isidoro, M. Ferrer, M. Perusquía, Vasoactive androgens: Vasorelaxing effects and their potential regulation of blood pressure, Endocr. Res. 43 (2018) 166–175.
[87] S.G. Chrysant, Controversies regarding the cardiovascular effects of testosterone replacement therapy in older men, Drugs Today (Barc) 54 (2018) 25–34.
[88] T.H. Jones, D.M. Kelly, Randomized controlled trials - mechanistic studies of testosterone and the cardiovascular system, Asian, J. Androl. 20 (2018) 120–130.
[89] M. Perusquía, Correlation between nongenomic action of C19-steroids and COVID-19 severity, Physiol. Res. 70 (2021) S135–S144.
[90] P. Yue, K. Chatterjee, C. Beale, P.A. Poole-Wilson, P. Collins, Testosterone relaxes rabbit coronary arteries and aorta, Circulation 91 (1995) 1154–1160.
[91] L.M. Montaño, E. Calixto, A. Figueroa, E. Flores-Soto, V. Carbajal, M. Perusquía, Relaxation of androgens on rat thoracic aorta: testosterone concentration dependent agonist/antagonist L-type Ca2+ channel activity, and 5beta-dihydrotestosterone restricted to L-type Ca2+ channel blockade, Endocrinology 149 (2008) 2517–2526.
[92] A.Q. Ding, J.N. Stallone, Testosterone-induced relaxation of rat aorta is androgen structure specific and involves K+ channel activation, J. Appl. Physiol. 91 (2001) 2742–2750.
[93] T.J. Costa, G.S. Ceravolo, R.A. dos Santos, M.A. de Oliveira, P.X. Araujo, L. R. Giaquinto, et al., Association of testosterone with estrogen abolishes the beneficial effects of estrogen treatment by increasing ROS generation in aorta endothelial cells, Am. J. Physiol. Heart Circ. Physiol. 308 (2015) H723–H732.
[94] M. Perusquía, R. Hernandez, M.A. Morales, M.G. Campos, C.M. Villalon, Role of endothelium in the vasodilating effect of progestins and androgens on the rat thoracic aorta, Gen. Pharmacol. 27 (1996) 181–185.
[95] M. Perusquía, C.M. Villalon, Possible role of Ca2+ channels in the vasodilating effect of 5beta-dihydrotestosterone in rat aorta, Eur. J. Pharmacol. 371 (1999) 169–178.
[96] V.P. Deenadayalu, R.E. White, J.N. Stallone, X. Gao, A.J. Garcia, Testosterone relaxes coronary arteries by opening the large-conductance, calcium-activated potassium channel, Am. J. Physiol. Heart Circ. Physiol. 281 (2001) H1720–H1727.
[97] R.D. Jones, K.M. English, T.H. Jones, K.S. Channer, Testosterone-induced coronary vasodilatation occurs via a non-genomic mechanism: evidence of a direct calcium antagonism action, Clin. Sci. (Lond) 107 (2004) 149–158.
[98] J.K. Crews, J.N. Herrington, J.P. Granger, R.A. Khalil, Decreased endothelium-dependent vascular relaxation during reduction of uterine perfusion pressure in pregnant rat, Hypertension 35 (2000) 367–372.
[99] S. Maynard, F.H. Epstein, S.A. Karumanchi, Preeclampsia and Angiogenic Imbalance, Annu. Rev. Med. 59 (2008) 61–78.
[100] L.C. Sanchez-Aranguren, C.E. Prada, C.E. Riano-Medina, M. Lopez, Endothelial dysfunction and preeclampsia: role of oxidative stress, Front. Physiol. 5 (2014) 372.
[101] Z.B. Tuzcu, E. Asicioglu, M. Sunbul, B. Ozben, H. Arikan, M. Koc, Circulating endothelial cell number and markers of endothelial dysfunction in previously preeclamptic women, Am. J. Obstet. Gynecol. 213 (2015) 533–537.
[102] A.H. Brandao, L.R. Felix, E.C. Patricio, H.V. Leite, A.C. Cabral, Difference of endothelial function during pregnancies as a method to predict preeclampsia, Arch. Gynecol. Obstet. 290 (2014) 471–477.
[103] P. Tep-areenan, D.A. Kendall, M.D. Randall, Testosterone-induced vasorelaxation in the rat mesenteric arterial bed is mediated predominantly via potassium channels, Br. J. Pharmacol. 135 (2002) 735–740.
[104] T.M. Chou, K. Sudhir, S.J. Hutchison, E. Ko, T.M. Amidon, P. Collins, Testosterone induces dilation of canine coronary conductance and resistance arteries in vivo, Circulation 94 (1996) 2614–2619.
[105] C. Molinari, A. Battaglia, E. Grossini, D.A. Mary, C. Vassanelli, G. Vacca, The effect of testosterone on regional blood flow in prepubertal anaesthetized pigs, J. Physiol. 543 (2002) 365–372.
[106] F.L. Wynne, R.A. Khalil, Testosterone and coronary vascular tone: implications in coronary artery disease, J. Endocrinol. Invest. 26 (2003) 181–186.
[107] J.K. Crews, R.A. Khalil, Gender-specific inhibition of Ca2+ entry mechanisms of arterial vasoconstriction by sex hormones, Clin. Exp. Pharmacol. Physiol. 26 (1999) 707–715.
[108] J. Hall, R.D. Jones, T.H. Jones, K.S. Channer, C. Peers, Selective inhibition of L-type Ca2+ channels in A7r5 cells by physiological levels of testosterone, Endocrinology 147 (2006) 2675–2680.
[109] J.L. Scragg, R.D. Jones, K.S. Channer, T.H. Jones, C. Peers, Testosterone is a potent inhibitor of L-type Ca(2+) channels, Biochem. Biophys. Res. Commun. 318 (2004) 503–506.
[110] J.L. Scragg, M.L. Dallas, C. Peers, Molecular requirements for L-type Ca2+ channel blockade by testosterone, Cell. Calcium 42 (2007) 11–15.
[111] M. Seyrek, O. Yildiz, H.B. Ulusoy, V. Yildirim, Testosterone relaxes isolated human radial artery by potassium channel opening action, J. Pharmacol. Sci. 103 (2007) 309–316.
[112] J.G. Murphy, R.A. Khalil, Decreased [Ca(2+)](i) during inhibition of coronary smooth muscle contraction by 17beta-estradiol, progesterone, and testosterone, J. Pharmacol. Exp. Ther. 291 (1999) 44–52.
[113] X. Qiao, K.R. McConnell, R.A. Khalil, Sex steroids and vascular responses in hypertension and aging, Gend. Med. 5 (2008) S46–S64.
[114] C.E. Costarella, J.N. Stallone, G.W. Rutecki, F.C. Whittier, Testosterone causes direct relaxation of rat thoracic aorta, J. Pharmacol. Exp. Ther. 277 (1996) 34–39.
[115] J. Navarro-Dorado, L.M. Orensanz, P. Recio, S. Bustamante, S. Benedito, A. C. Martínez, Mechanisms involved in testosterone-induced vasodilatation in pig prostatic small arteries, Life Sci. 83 (2008) 569–573.
[116] K. Iki, I. Miyamori, H. Hatakeyama, T. Yoneda, Y. Takeda, R. Takeda, The activities of 5 beta-reductase and 11 beta-hydroxysteroid dehydrogenase in essential hypertension, Steroids 59 (1994) 656–660.