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ORIGINAL ARTICLE

Hypotestosteronemia is an important factor for the development of hypertension: elevated blood pressure in orchidectomized conscious rats is reversed by different androgens

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Abstract

Purpose Hypotestosteronemia is an aging-associated disease. Little is known about experimental evidence linking androgen deficiency to hypertension. Various androgens are acute vasodilators, both in vitro and in vivo. We aimed to systematically investigate blood pressure (BP) in male normotensive intact or orchidectomized (ORX) Wistar and Wistar-Kyoto rats. Furthermore, we studied the acute antihypertensive responses of testosterone (TES), its precursor (DHEA), or its 5β-reduced metabolite (5β-DHT) in conscious, unrestrained, hypertensive Wistar rats caused by orchidectomy to determine their potency and efficacy. Similarly, the mechanism of their action mediated by nitric oxide (NO) was studied in vivo.

Methods BP of ORX rats was evaluated weekly for 18 weeks by tail cuff plethysmography. Subsequently, BP of ORX Wistar rats was measured by chronic indwelling vascular catheters, arterial, and venous catheters were implanted under anesthesia for BP recording and androgen administration, respectively. Then, a dose–response curve of each androgen was performed. Likewise, the dose–response curve of 5β-DHT, the most potent androgen, was repeated in the presence of a nonselective NO synthase inhibitor (L-NAME) or an inhibitor of endothelial NO synthesis (Endothelin-1).

Results ORX rats progressively increased systolic/diastolic BP (167 ± 2.8/141 ± 3.3 mmHg) over 18 weeks. No difference was found between strains. The BP was reduced in a dose-dependent manner caused by i.v. bolus injection of each androgen, with a rank order of potency of: 5β-DHT = DHEA>>TES. Dose-dependent antihypertension induced by 5β-DHT in ORX rats was not abolished in the presence of L-NAME or Endothelin-1.

Conclusions These in vivo experimental findings reveal that hypotestosteronemia is a determining factor for the development of hypertension which is powerfully reduced by androgen administration, and 5β-DHT induces a potent and effective antihypertensive response by a NO-independent mechanism.

Keywords Androgens · 5β-dihydrotestosterone · Androgen-regulated blood pressure · Antihypertensive response · Androgen deficiency · Hypertension in aging men

Introduction

Cardiovascular diseases continue to increase worldwide, a phenomenon that is particularly problematic among the aging population. One of the most relevant cardiovascular diseases is hypertension and is clearly a public global health challenge. In this context, large epidemiological and observational studies report that androgen deficiency is the most widely recognized and evaluated hormonal alteration associated with male aging. Following this logic, it has been hypothesized that testosterone (TES) deficiency could be a catalyst for hypertension [1–7]. Such lines of evidence have indicated that hypotestosteronemia is indeed an important factor for the development of hypertension [8]. However, little is known about experimental evidence linking androgen deficiency to hypertension.

Our recent in vivo experimental contributions in animals have reported that blood pressure (BP) regulation is expected as a consequence of androgen-induced vasorelaxation [9–11]. In this respect, we have first reported that

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some metabolites of TES, are capable of blocking the vasopressor responses to noradrenaline or a Ca^{2+} channel agonist in anesthetized vagosympathectomized, pithed Wistar male rats [12]. Subsequently, we documented that TES and its 5β-reduced metabolite (5β-dihydrotestosterone, 5β-DHT) also induce a marked reduction of BP in both conscious normotensive Sprague-Dawley and testicular feminized male rats (Tfm; androgen receptor-deficient) [9].

Remarkably, androgens can also elicit a significant antihypertensive responses in conscious spontaneously hypertensive male rats (SHR), and TES deprivation by orchidectomy increases BP in normotensive Wistar-Kyoto (WKY) rats which was prevented by TES replacement therapy [10]. More recently, it was demonstrated that in an in vivo rat model of preeclampsia, the elevated mean arterial BP (MAP) was reduced significantly by dehydroepiandrosterone (DHEA), TES and its 5-reduced metabolites (5α- and 5β-DHT) [11]. In parallel, these findings have also shown that DHEA and 5β-DHT are greatly more potent than TES to evoke an antihypertensive response.

On the basis that castration of male rats is an accepted model for the study of androgen deficiency [13–15], this study systematically investigated hemodynamics in this experimental model; a general contribution to the knowledge of hypertension in aging men. Particularly, in this study we also analyzed the potential acute antihypertensive responses in conscious hypertensive rats caused by orchidectomy of different vasoactive androgens, such as DHEA, TES, and 5β-DHT which have been previously reported as potent antihypertensives in SHR and preeclamptic rats [10, 11]. Likewise, for the first time in vivo, we investigated the role of nitric oxide (NO), which may play a role in the mechanism by which androgens determine their antihypertensive response. With this in mind, the role of the endothelial NO in androgen-induced vasorelaxation in isolated vascular beds has been a matter of debate and remains controversial due to the fact that some studies have demonstrated that TES-induced vasorelaxation is inhibited by treatment with L-NAME [16–19], while some others have documented a NO-independent mechanism [12, 20, 21].

Materials and methods

Animals

The study was conducted in the Department of Cell Biology and Physiology, Institute for Biomedical Research, National Autonomous University of Mexico (UNAM). Animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication 86-23, revised 2014). Male Wistar and WKY rats, 18–21 weeks old age (body weight, range 250–300 g) were obtained from the Animal Center of the Institute for Biomedical Research and Institute of Cell Physiology, UNAM, respectively. Rats were housed in a standard experimental laboratory rodent housing for 2 weeks prior to the start of the experiments. All rats were randomly divided into four groups: orchiectomized (ORX) Wistar rats, intact Wistar rats (control), ORX WKY rats, and intact WKY rats (control).

Castration of male rats

In order to detect a major predisposition of WKY strain to develop hypertension to which spontaneously hypertensive rats (SHR) are genetically related, both Wistar and WKY rats were compared. Rats underwent a bilateral orchiectomy surgery, and others intact rats were controls. Briefly, the rats were anesthetized with 80/10 mg/kg of Ketamine/Xylazine i.p. Each testis was excised through a small incision at the posterior end of the scrotum, the spermatic cord was then ligated with silk suture and transected distal to the ligature to remove the testis. The transected cord was allowed to retract into the inguinal canal and the scrotum was then closed with silk suture. Then, changes in BP followed weekly during the 18 weeks following orchiectomy surgery of ORX groups, as well as the controls (intact rats). Systolic/diastolic and mean arterial blood pressure (MAP) was measured before orchidectomy (day 0) and every week during the next 18 weeks with an indirect method, by the same researcher, in a conscious and slightly restrained rat by tail cuff plethysmography using a small animal tail-cuff BP system (LE 5002 storage pressure; Panlab Harvard Apparatus, Spain). For these measurements, rats were conditioned to minimal restraint in a warming chamber for 20 min/day for at least 3 days prior to initiating weekly BP measurement. The warming chamber was kept at 32 °C; and while one rat was tested, another rat was being warmed in advance. After 5–10 min of stabilization in the chamber, a typical run involved five repetitions of the automated inflation–deflation cycle. The mean of five readings within a 2–5 mmHg rang was recorded as the BP for each animal.

Chronic indwelling vascular catheters

On the basis that the development of hypertension by orchiectomy did not show any difference between Wistar and WKY rats, only Wistar rats were used after 18 weeks, the ORX rats were anesthetized with a combination of Ketamine (80 mg/kg) and Xylazine (10 mg/kg) given intraperitoneally, and chronic indwelling catheters were placed in the left carotid artery and right jugular vein, using polyurethane tubing (internal diameter 0.36 mm, external diameter 0.84 mm, "Microrenathane", Braintree Scientific, Inc., USA). The catheters were passed subcutaneously to the dorsal surface of

the neck and exteriorized, and the rats were then fitted with polyester cloth vests with Velcro closures that surrounded the chest and neck to protect the catheters.

Evaluation of hemodynamic data

After the ORX rats had completely recovered from anesthesia and catheter implant surgery (24–48 h), they were placed in clear plexiglass rodent restrainers, which allowed some free movement, and allowed to acclimate to experimental conditions for 90 min. The carotid arterial catheter was connected to a pressure transducer (Grass P23 XL) adapted to an MP150 Research System (Biopac Systems Inc., CA), and the output combined with AcqKnowledge software for data acquisition and analysis. When hemodynamic variables had been stable for a period of at least 60 min, baseline values of BP, i.e., systolic BP (SBP), diastolic BP (DBP), and MAP in mmHg and heart rate (HR) in beats per minute (beats min^{-1}, BPM) were monitored continuously during the experiment and calculated in the AcqKnowledge software. In all groups, the recording continued for at least 90 min after the highest dose of androgen had been administered.

Response induced by androgens in hypertensive rats by orchiectomy

After a 60–90-min stabilization period (baseline values), dose–response curves were obtained from ORX rats for each androgen (DHEA, TES or 5β-DHT; only a single androgen was tested in each animal). Androgens were administered intravenously by bolus injected (through the jugular vein catheter) cumulatively at log doses of: −1.0, 0.0, 1.0, and 2.0 log μmol kg^{-1} min^{-1}, doses used according to our previous reports [10, 11], which were administered over a one-minute period by means of a digital programmable single-syringe pump (KD Scientific, MA; Kds 100) with an interval of ~20 min between each dose. The hemodynamic values after each dose were compared with the baseline values to report: (a) the difference in mmHg of BP (ΔmmHg) and (b) the changes in HR (ΔBPM).The cumulative dose–response curves for each androgen in ORX rats were determined independently. The potency of each androgen was evaluated by calculation of the effective dose 50 (ED_{50}). The antihypertensive efficacy of each androgen was evaluated as the maximal effect (E_{max}) for each androgen. The ED_{50} and E_{max} values for each androgen were compared statistically.

Androgen-induced BP reduction in the presence of L-NAME or Endothelin-1 (ET-1)

In different ORX Wistar rats, by using the same invasive method: (i) 10 μmol kg^{-1} min^{-1} L-NAME (nonselective NO synthase inhibitor; *N*-ω-nitro-L-arginine methyl ester) this dose causes a pressor response in BP [22]; or (ii) 100 nmol kg^{-1} min^{-1} ET-1 (inhibitor of endothelial NO synthesis; ET-1), this dose was chosen on the basis of previous experiments and we determined, to a wide range of doses (1, 10, 100 nmol kg^{-1} min^{-1} ET-1), that at 100 nmol kg^{-1} min^{-1} ET-1 increases the BP significantly in conscious rats. Each drug was given i.v., by bolus injected through the jugular vein catheter, independently to different ORX rats after the usual stabilization period (60–90 min). When the pressor response induced by L-NAME or ET-1 attained a plateau, the most potent and effective antihypertensive androgen, 5β-DHT (selected from the dose–response experiments), which is genomically inactive and remarkably it is not aromatizable and exhibits the highest efficacy and potency for reduction of systemic BP, was administrated by i.v. bolus injected in the same range of dose as in the dose–response experiments.

Control experiments with the vehicles used

After the stabilization period, in order to compare the response of androgens and its vehicle (ETOH), separated vehicle-controls were run for ORX rats with i.v. bolus injection of the same volume of vehicle, absolute ethanol (ETOH; all doses administered never exceeded 0.5 mL ETOH in each rat) at identical time intervals as the androgens. The same was performed for vehicle of L-NAME or ET-1 (0.08 mL distilled water, respectively).

Data presentation and statistical analysis

Data are expressed as the means ± SD ($n = 6$, number of animals). In the text and figures, changes in BP and HR in vivo were expressed as the change from baseline (mmHg) or beats per minute (Beats min^{-1}, BPM), correspondingly. The antihypertensive potency of each androgen in vivo was evaluated by calculation of the effective dose 50 (ED_{50} = dose of androgen required to reduce BP by 50% of the maximal response compared to control BP). The ED_{50} was calculated by linear regression from the cumulative dose–response curve obtained from each animal. The antihypertensive efficacy of each androgen was evaluated by calculation of the maximal effect (E_{max}; antihypertensive response at 2.0 log μmol kg^{-1} min^{-1}). Nonpaired Student *t*-tests were used to compare the responses between any two groups. To compare E_{max} or ED_{50} values in BP studies, a two-way ANOVA followed by Tukey's test were used. Significance was accepted at $p < 0.05$.

Drugs and chemicals

With exception of ketamine (Pisa Farmaceutica, Mexico), xylazine (Bayer, Germany), and 17β-hydroxy-5β-androstan-3-one (5β-dihydrotestosterone; 5β-DHT) (Steraloids, Inc., Newport, RI), all other compounds were purchased from

Sigma (St. Louis, MO) including: DHEA (3β-hydroxy-5-androsten-17-one), TES (17β-hydroxy-4-andosten-3-one), L-NAME, and ET-1. Each androgen was prepared as a stock solution in absolute ETOH and then diluted in ETOH to the dose needed for each experiment. The remaining drugs were dissolved in distilled water.

Results

Development of hypertension by orchidectomy

Figure 1a shows that ORX rats displayed a progressive increase (time-dependent response) in MAP to a maximum of 151 ± 2.1 mmHg and SBP/DBP 167 ± 2.8/141 ± 3.3 mmHg at 18 weeks after ochidectomy, which was significantly different than SBP/DBP (119 ± 1.8/97 ± 2.6 mmHg) and MAP (110 ± 1.8 mmHg) of rats prior to orchidectomy at week "0"; the progressive increase in SBP/DBP and MAP of ORX rats achieved statistical significance ($p < 0.0001$) at 5 weeks after orchidectomy. No significant difference was observed in BP from week 11 to week 18. In contrast, the intact rats, without orchidectomy, did not increase BP during 18 weeks and these values were significantly different ($p < 0.0001$) vs. ORX rats. We did not observe any significant difference between Wistar and WKY rats in the development of hypertension induced by orchidectomy over 18 weeks (Fig. 1b), for this reason, in the subsequent experiments only the ORX Wistar rats were used.

Response induced by androgen in high BP of ORX rats

All androgens caused an immediate (~2 min) fall in BP. As shown in Fig. 2, the i.v. bolus injection of each androgen at

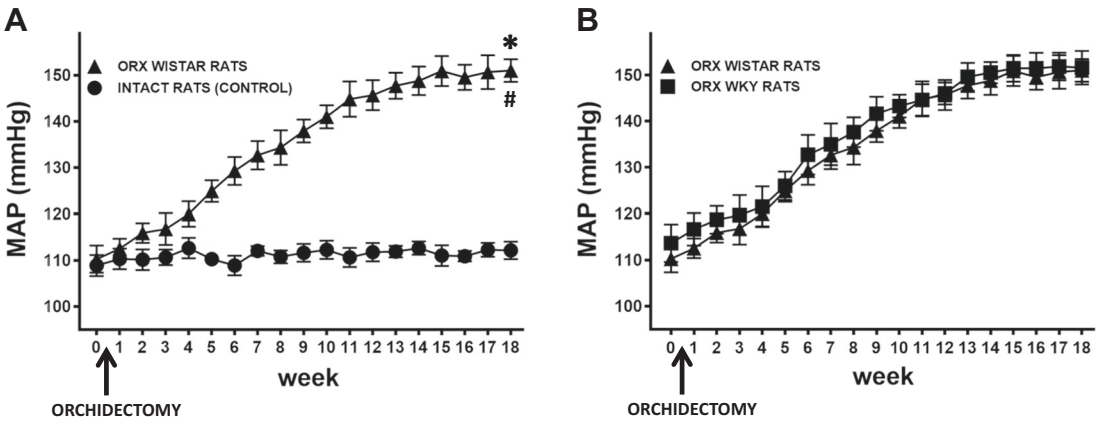

Fig. 1 Mean arterial blood pressure (MAP) in male normotensive intact (control) or orchidectomized (ORX) Wistar and Wistar-Kyoto (WKY) rats. MAP was gradually increased after orchidectomy. **a** Statistical significance between curves of ORX rats and intact rats $^{\#}p <$ 0.0001; MAP values are different $^{*}p < 0.0001$ before orchidectomy (week 0) as compared with week 18 after orchidectomy. **b** No difference was found between time-dependent curve between ORX Wistar and ORX WKY rats. Arrow indicates the orchydectomy time. Each symbol represents the mean ± SD from six animals

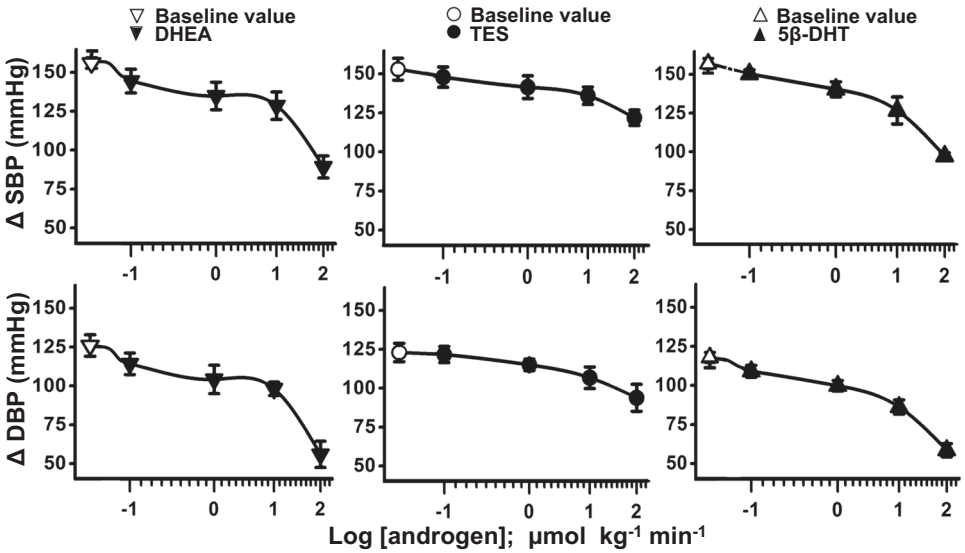

Fig. 2 Androgens reduce arterial blood pressure (BP) in conscious ORX Wistar rats. Dose-dependent changes in BP, systolic (SBP), and diastolic (DBP), induced by increasing cumulative doses of each androgen. Data are mean ± SD, $n = 6$ in each group

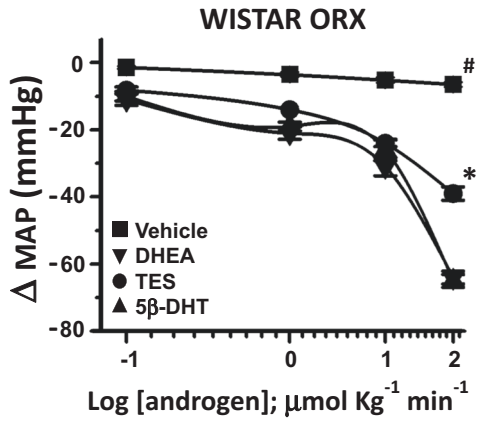

Fig. 3 Dose-dependent changes on mean arterial blood pressure (MAP) elicited by each androgen in conscious ORX Wistar. The vehicle (absolute ethanol at equivalent volume of all doses administered) did not alter MAP significantly ($p > 0.5$), but the changes in MAP produced by each androgen were significantly different from the vehicle-control ($^{\#}p < 0.01$). DHEA and 5β-DHT curves did not differ ($p > 0.05$), while these curves were significantly different compared with TES curve ($^{*}p < 0.05$). The E_{max} values of DHEA and 5β-DHT were significantly different to E_{max} of TES ($^{*}p < 0.05$)

cumulative doses produced dose-dependent reduction in BP (SBP/DBP) until normotensive levels to TES, while BP continued to decline until attaining hypotensive levels after the last dose to DHEA (89 ± 7.1/56 ± 8.4 mmHg) and to 5β-DHT (93 ± 2.0/58 ± 4.1 mmHg). Figure 3 summarizes the reduction of BP (MAP values) by the all androgens tested; the vehicle-control did not significantly alter BP but the response induced by each androgen was significantly different from the vehicle-control at all doses tested ($p < 0.01$). HR significantly declined only at the highest dose to DHEA or 5β-DHT ($p < 0.01$) but was not altered by TES (data no shown).

Comparison of antihypertensive potency of each androgen

The analysis of the curves shows that no difference was observed in the antihypertensive potency (ED_{50}) of 5β-DHT of TES and DHEA (precursor of TES), which were equipotent between them, and remarkably they were 2.1 and 2.0 times more potent, respectively, than that of TES; thus, the rank order of antihypertensive potency was: 5β-DHT = DHEA>>TES. Likewise, the antihypertensive efficacy (E_{max}) was: 5β-DHT = DHEA>>TES, values are shown in Table 1.

The potential role of NO in androgen-induced reduction in BP

After 18 weeks of orchidectomy, in separate ORX Wistar rats, we observed that the bolus injection of L-NAME

Table 1 Androgen-reduced blood pressure in conscious orchidectomized Wistar rats

Androgen	ED_{50} log µmol kg^{-1} min^{-1}	E_{max} (ΔMAP mmHg)	*r*	Potency[a]
DHEA	1.7 ± 0.2	−65 ± 1.9	0.86	2.0
TES	3.4 ± 0.2**	−39 ± 2.2*	0.96	1.0
5β-DHT	1.6 ± 0.1	−64 ± 1.9	0.90	2.1

Half-maximal reduction (ED_{50}) and maximal reduction (E_{max}) values of mean arterial blood pressure (MAP). The values are mean ($n = 6 \pm$ SD), n = number of animals. ED_{50} = value of androgen dose (log µmol kg^{-1} min^{-1}) required to inhibit 50% of MAP. ED_{50} was calculated by straight-line regression from every cumulative dose–response curve. E_{max}, change in MAP (-mmHg) at the highest dose. Pearson's correlation coefficient (*r*) represents the fitness of the straight line

$^{*}p < 0.05$, as compared to DHEA or 5β-DHT

$^{**}p < 0.01$, as compared to DHEA or 5β-DHT

[a]Potency was calculated from ED50 values by the formula: ED_{50} TES/ED_{50} 5β-DHT or; ED_{50} TES/ED_{50} DHEA assuming a value of 1.0 to TES

potencies significantly ($p < 0.05$) elevated BP by orchidectomy, which means that basal MAP (135 ± 5.2 mmHg) was elevated significantly following bolus administration of L-NAME (164 ± 3.5 mmHg) (Fig. 4a). Interestingly, 5β-DHT at −1.0, 0.0, 1.0, and 2.0 log µmol kg^{-1} min^{-1} inhibited, in a dose–response manner, the pressor response to L-NAME (Figs. 4a and 5). We observed that this dose–antihypertensive response curve to 5β-DHT was not abolished in the presence of L-NAME but potentiated at the last two doses administrated (Fig. 5).

Similarly, administration of ET-1 results in a biphasic response characterized by a transient depressor response, followed by a pronounced and persistent hypertension (MAP; 134 ± 7.3 vs. 151 ± 9.1 mmHg), which was effectively inhibited in a dose–response manner by the different doses of 5β-DHT (Fig. 4b), resulting an identical dose–antihypertensive response to 5β-DHT in the presence of ET-1 (Fig. 5).

Outstandingly, these in vivo experiments display that L-NAME or ET-1 did not eliminate the dose-dependent antihypertensive response to 5β-DHT.

Discussion

General

Androgen deficiency regarding arterial BP is unclear. Our aim was to determine whether androgen deficiency caused by orchidectomy in rats as an experimental model of androgen deficiency in aging men [13–15], may cause high BP. In the present study, we observed that the decrease of androgens by orchidectomy progressively raised BP

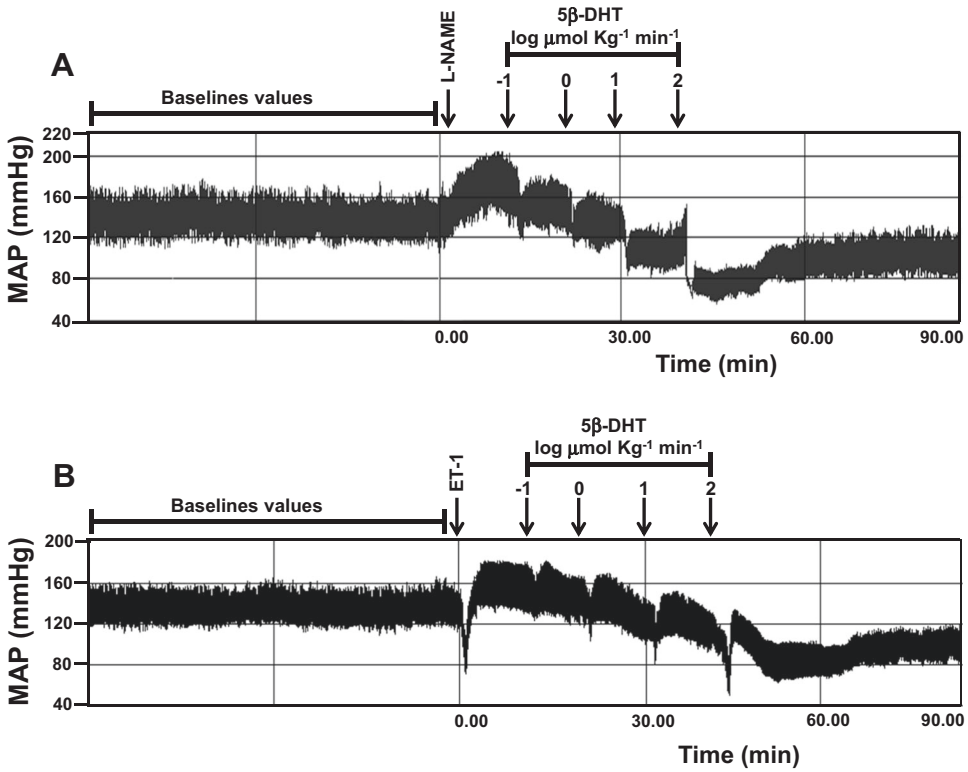

Fig. 4 Representative recordings of mean arterial blood pressure (MAP) in conscious ORX Wistar rats. Antihypertensive response elicited at different bolus i.v. injection of 5β-DHT in the presence of: **a** 10 µmol kg^{-1} min^{-1} L-NAME or; **b** 100 nmol kg^{-1} min^{-1} ET-1. The arrows indicate the times of i.v. drug injection

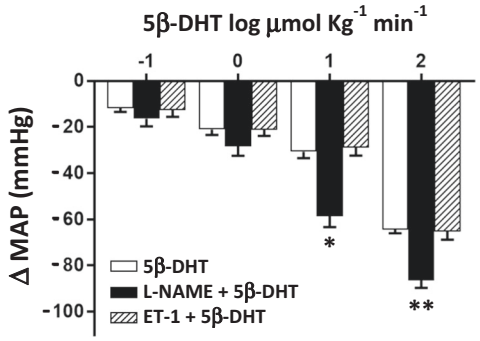

Fig. 5 Maximal changes in mean arterial blood pressure (MAP) in conscious hypertensive Wistar rats caused by orchidectomy at 18 weeks. Dose–response antihypertensive curve to 5β-DHT in the absence and in the presence of L-NAME at 10 µmol kg^{-1} min^{-1} or ET-1 at 100 nmol kg^{-1} min^{-1}. Androgen-reduced MAP with L-NAME pretreatment was significantly potentiated at the last two doses of androgen *$p < 0.01$; **$p < 0.05$. Data are mean ± SD, $n = 6$ rats per bar

reaching high levels of hypertension (167 ± 2.8/141 ± 3.3 mmHg). Previously, we have observed that androgen deprivation by surgical castration of WKY rats increases the arterial BP in a short time of 10 weeks, which can be entirely prevented by TES replacement [10]. Our present study focused on the changes of BP over a longer period of time (18 weeks) after orchidectomy in two different strains. When compared to week 10 [10], BP levels significantly increased at 18 weeks (present data). A time-dependent increment of BP was observed in ORX rats in both Wistar and WKY rats. This datum correlates with the fact that as men get older, the level of TES in the body gradually becomes lower, which may lead to a number of health problems, including hypertension. In this respect, it has been widely demonstrated that the natural aging process estimates that TES decreases by about 10% every decade after men reach the age of 30. Consequently, this experimental study, in a model of androgen deficiency in aging men, indicates that no doubt exist to conclude that hypertension is associated with androgen deficiency, thus explaining the critical role that endogenous androgens may play in the maintenance of normal BP. In contrast, several laboratories have reported findings contradictory to those of the present study; thus, a study [23] reported that high salt diet-induced hypertension in Sprague-Dawley rats was reversed by orchidectomy, while TES replacement prevented the reversal. Loh and Salleh [24] also reported that MAP and plasma TES levels decreased by orchidectomy. Castration of Wistar rats did not alter SBP while treatment with TES did increase BP [25]. On the contrary, orchidectomy of SHR and WKY rats progressively raised MAP, which was prevented by TES treatment [10]. One possible explanation for these contradictory findings might be the experimental conditions, such as animal age, and differences in experimental methods including use of anesthetized rats with spontaneous ventilation, compared with conscious rats used in the present study. In this context, studies in anesthetized animals might sound similar to

in vivo conscious animals but the anesthetic effect and the damage caused by tracheostomy may confound the results.

In line with our findings, it has been hypothesized that androgen levels are actually decreased in men with chronic diseases, such as hypertension, obesity, heart diseases, chronic kidney disease, and metabolic syndrome [26, 27]. In the present work we tested the hypothesis that low androgen levels are associated to hypertension in older men. Admittedly, the development and progression of hypertension are certainly multifactorial but may be explained, at least in part, by lifetime patterns of sex hormone deficiency in older men. In the present study, we did not measure plasma levels of androgens due to the fact that this matter is out of the scope of the present paper. To justify this, it is also important to take into account the earlier findings of Coyotupa et al. [13], who reported that orchidectomy drastically decreased the physiological levels of TES and 5α-DHT in rats, as well as a recent study indicated that orchidectomy rapidly reduced plasma total TES concentrations [14].

Androgen-induced BP reduction and the chemical structure relationship

Each androgen selected was capable of reducing of high BP caused by orchidectomy in a dose–response manner, which suggests that they may be important regulators of BP and may also be of value for therapeutic use. Recently, we have reported that these androgens are capable of inducing an acute reduction in BP in conscious normotensive rats [9], as well as in males and females models of hypertension, such as: conscious SHR male rats [10] and preeclamptic female rats [11]. To our knowledge, the present study is novel due to the fact that this is the first time it is reported that the same androgens are also capable of reducing high BP in a hypertensive model of the aging male.

The results of this study indicate that the antihypertensive potency of each androgen was different. TES turned out to be two times less potent than its precursor (DHEA), as well as its 5β-DHT, both of which were equipotent. Likewise, TES can reduce BP until normotensive values while DHEA and 5β-DHT reduce BP until hypotensive BP values. These findings could apparently indicate that TES is a better candidate in the therapeutic use to prevent hypertension; however, its low potency implies its use at high doses. In this respect, before considering that TES is a good alternative for androgen replacement therapy, it is very important to ponder that TES is biotranformed: (i) into estrogens by aromatization (with estrogenic properties); and (ii) into its five-reduced metabolites, of which its 5α-reduced metabolite (5α-DHT) has high androgenic properties and is associated with prostate cancer. In the case of DHEA, it is important to consider that this hormone is a precursor of steroids and is also obviously biotransformed into active estrogens and androgens. In marked contrast, the 5β-reduced metabolite of TES, 5β-DHT, indeed offers potential therapeutic benefits in the treatment of hypertension as a result of several qualities: this metabolite is highly potent to reduce BP, it is genomically inactive, does not have androgenic properties, and it is a nonaromatizable androgen without estrogenic properties. Taken together our analysis indicates that 5β-DHT is the best choice for its potential therapeutic use, either for the prevention hypertension or for acute hypertensive emergencies in aging men, and perhaps it could be utilized for overall androgen replacement therapy as well. In agreement with this suggestion, some isolated clinical studies have reported that TES replacement therapy, but not 5β-DHT replacement therapy, reduced DBP and improved serum lipid profile in aged hypogonadal men [28] and some collateral findings described that TES replacement therapy is capable of reducing systolic and DBP [29–32]. Admittedly, further clinical studies are needed with 5β-DHT.

On the other hand, the different potency of each androgens studied has been attributed to the marked differences in their three-dimensional molecular structure. The high potency of 5β-DHT could be due to the dramatic bend at the A/B-ring junction in its steroidal structure, while the Δ4,3-keto structure of TES and 3β-hydroxy-Δ5 structure of DHEA are planar structures. Notably, DHEA also has the inclusion of a 17-keto group which may be relevant to increase its efficacy.

Certainly, TES is rapidly metabolized in vivo (≥10 min) but is slower when this androgen is catalyzed by the enzyme 5β-reductase into 5β-DHT. Thus, since the rapid time-frame (1–2 min) of the antihypertensive responses induced by androgens, we ruled out the in vivo bioconversion of androgens.

Mechanism of androgen-induced antihypertensive action

It has been widely documented that androgens regulate the cardiovascular system through both genomic (anti-inflammatory activity) [33] and nongenomic (vasorelaxing effect in isolated blood vessels from several species) [34–37] mechanisms. In addition, androgen-regulated BP is characterized as a nongenomic action, since the antihypertension caused by androgens is immediate to their i.v. bolus administration, as well as to their hypotensive response elicited in Tfm rats (male rats with androgen receptor deficiency) [9–11]. Such findings have categorically shown that androgen-induced BP reduction is nongenomically mediated.

The in vitro vasorelaxing response of androgens and the role that the endothelium plays has been largely

controversial. Some reports have documented that TES-induced vasorelaxation is inhibited by treatment with L-NAME [16–19], while some others have revealed a NO-independent mechanism [12, 20, 21]. However, it is noteworthy to mention that none of these studies were performed in conscious rats.

Consequently, the potential role of NO in androgen-induced reduction in BP was explored in vivo. It is important to take into account that the vascular endothelium exerts important effects on vascular tone through the release of vasodilator (endothelium-derived relaxing factor) [38] and vasoconstrictor molecules. NO liberated from endothelial cells in response to chemical or physical stimuli exerts vasodilatation, decreases vascular resistance, increases blood flow, and lowers systemic blood pressure, and assists in thrombosis prevention [39]. ET-1, also liberated from the endothelium, acts as a counteracting molecule against NO via interfering with NO synthesis and thus observing its vasoconstrictor property [40].

Our data in conscious ORX hypertensive rats revealed that: (i) the blockade of NO synthase by L-NAME did not alter the antihypertensive response to 5β-DHT; and (ii) ET-1, by binding to ET_A receptors interferes with NO synthesis and acts to counter NO-induced vasodilatation [40], and under these circumstances, we observed that the antihypertensive response to 5β-DHT did not change on the pressor response to ET-1.

It is relevant to comment that the antihypertensive response induced by 5β-DHT at the last two doses in the presence of L-NAME was potentiated. Consistent with this result, we have previously found that when the BP is very high, the androgen response is potentiated [10, 11].

Regarding the results of the present work, it is possible to exclude the involvement of a putative NO-related pathway in the establishment of antihypertensive response induced by androgens, which in turn also reinforces the evidence that androgens act on different sites of action, resulting in non-genomic vasorelaxation and antihypertension. It has been reported that androgens are blocking external Ca^{2+} influx through L-type Ca^{2+} channels (L-VOCC). Our research group has recently reported that 5β-DHT abolished the pressor response to a Ca^{2+} agonist (Bay K 8644) in conscious SHR rats suggesting a blockade of Ca^{2+} entry through L-VOCC in vivo [10]. These results are entirely consistent with previous in vitro [21, 41–44] and in single vascular myocytes [45–48] reports. Taken together these lines of experimental evidence, the nongenomic antihypertensive mechanism of androgens appears to preferentially block extracellular Ca^{2+} influx through L-VOCCs.

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Author contributions M.P., N.H., D.C. were involved of acquisition data. M.P. conceived and designed the study, analyzed and interpreted the data, and drafted the article also. All authors contributed to the revising of the manuscript, and approved the final version of this manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the Institute for Biomedical Research, UNAM.

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