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Possible role of Ca^{2+} channels in the vasodilating effect of 5β-dihydrotestosterone in rat aorta

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Abstract

It has previously been shown that the androgen, 5β-dihydrotestosterone (17β-hydroxy-5β-androstan-3-one, 5β-DHT), is able to produce an endothelium-independent vasodilating effect in rat aorta. The present study analyzed the mechanisms underlying the above vasodilator effect of 5β-dihydrotestosterone, with particular emphasis on verifying a possible interaction with $GABA_A$ receptors, β-adrenoceptors and Ca^{2+} channels. Rat aortic rings without endothelium were isometrically recorded. 5β-Dihydrotestosterone produced a concentration-dependent relaxation on the contractions induced by noradrenaline (NA; 0.3 μM) or K^+ (KCl; 60 mM), with the latter being more sensitive to 5β-dihydrotestosterone-induced relaxation than the former; the concentration–response curves showed that 5β-dihydrotestosterone is significantly more potent than 17β-estradiol (1,3,5(10)-estratrien-3,17β-diol) to induce vasodilatation. The vasodilating effect of 5β-dihydrotestosterone on noradrenaline-induced contraction was resistant to blockade by the $GABA_A$ receptor antagonists, picrotoxin or bicuculline, and the β-adrenoceptor antagonist, propranolol, a finding that excludes an interaction of the steroid with $GABA_A$ receptors and β-adrenoceptors. Interestingly, the contractions evoked by calcium in depolarized tissues were substantially inhibited by 5β-dihydrotestosterone, implying that this steroid could be an endogenous calcium channel blocker; consistent with this finding, 5β-dihydrotestosterone was able to relax tissues precontracted with the calcium channel opener, Bay K 8644. Moreover, although the rings precontracted with noradrenaline and potassium were almost equipotently relaxed by 5β-dihydrotestosterone. Nifedipine was more potent than 5β-dihydrotestosterone to block the potassium-induced contraction, but the steroid was more effective than nifedipine to prevent noradrenaline-induced contraction. The above results suggest that 5β-dihydrotestosterone causes relaxation of rat aorta by acting directly on the membrane of smooth muscle cells; this non-genomic action may be explained in terms of a blockade of voltage- and receptor-dependent calcium channels, a mechanism that restricts the availability of extracellular calcium in the contractile machinery.

Keywords: Steroids action; Androgen; Dihydrotestosterone; Vasorelaxation; Ca^{2+} channel antagonism; Thoracic aorta

1. Introduction

It has been shown that sex steroid hormones, including 5-reduced progestins and androgens, are able to produce, by a non-genomic effect, relaxation of several smooth muscle preparations (Kubli-Garfias, 1987; Kubli-Garfias et al., 1987; Perusquía et al., 1990, 1997), including the isolated rat thoracic aorta (Perusquía et al., 1996; Rodríguez et al., 1996). Indeed, ovarian steroids are vasoactive substances that may produce: (i) hypotension in several species, including humans (e.g., Magness and Rosenfeld, 1989; Elkayam, 1992; Vargas et al., 1995); and (ii) blockade of the vasopressor responses induced by noradrenaline in rats (Kondo et al., 1980; Cheng and Gruetter, 1992; Shan et al., 1994) or by angiotensin II in ovariectomized rabbits with prolonged estradiol treatment (Yoshimura et al., 1984). However, little attention has been paid to the potential effects of male sex hormones (androgens) in vascular smooth muscle. In this respect, we have recently shown that 5-reduced androgens are able to produce an endothelium-independent vasodilating effect on rat thoracic aorta (Perusquía et al., 1996). Notably, this study showed 5β-dihydrotestosterone (5β-DHT) as the most potent vasorelaxant steroid, even compared to the vasodilator response by progestins.

On this basis, the present study was designed to analyze the mechanisms underlying the endothelium-independent

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vasodilator effect of 5β-dihydrotestosterone in rings of rat thoracic aorta, with particular emphasis on verifying a possible interaction with $GABA_A$ receptors, β-adrenoceptors and calcium channels (voltage- and receptor-operated).

2. Materials and methods

2.1. Animals

Experiments were carried out in male adult Wistar normotensive rats (200–250 g). The animals were maintained at a 12/12-h light–dark cycle, with light beginning at 0700. The rats were kept in a special room at constant temperature (22 ± 2°C) and humidity (50%), with food and water ad libitum. The present project was approved by our Animal Care Committee, and experiments were conducted in accordance with the published *Guiding Principles in the Care and Use of Animals* approved by the American Physiological Society.

2.2. Vascular tension studies

Male Wistar rats were killed by cervical dislocation. The descending thoracic aorta was then removed and placed in a Krebs-Ringer bicarbonate solution with the following composition (in mM): $NaHCO_3$ 24.9, NaCl 118.5, KCl 4.74, KH_2PO_4 1.18, $MgSO_4$ 1.18, $CaCl_2$ 2.5 and glucose 12.0. The aorta was cleaned of fat, blood and connective tissue and cut into sectional rings of 1 cm in length. The endothelial layer was systematically removed, as previously described (Perusquía et al., 1996) and each ring was mounted horizontally in a temperature-controlled (37°C) organ bath containing 10 ml Krebs solution. The solution was continuously bubbled with 95% O_2–5% CO_2, resulting in a pH of 7.4. The tissues were attached to a tension transducer (Grass FT03C) connected to a polygraph (Grass 79). The rings were equilibrated for 1 h under a resting tension of 1 g (10 mN force) before use. After the equilibration period, tension generation was tested three times with noradrenaline 0.3 μM (dissolved in distilled water), and the response was recorded during 30 min, the rings were washed three times every 15 min between each noradrenaline response. Tissues with reproducible contractions were used for the study. In the absence of endothelium, acetylcholine 20 μM (dissolved in distilled water) was found to have no effect on such contractions (data not shown).

The relaxing effect of 5β-dihydrotestosterone was studied on a next tonic contraction produced by 0.3 μM noradrenaline or on the contraction induced by high potassium (KCl 60 mM), which was elicited for substitution of high $[K^+]$ solution (KCl 60 and NaCl 64.7 mM). The KCl-induced contraction was also repeated three times before the 5β-dihydrotestosterone addition. Non-cumulative concentrations (from 7.5 to 120 μM) of 5β-dihydrotestosterone were applied during the sustained phase of tension generated by the vasoconstrictor agents (10 min before any contraction had been induced). In order to evaluate the relaxing response of 5β-dihydrotestosterone, its potency was compared with that of 17β-estradiol, which was used as a positive control under the same experimental conditions. All steroids were dissolved in ethanol and each concentration was always applied at 0.1% (17.14 mM). Due to the insolubility of steroids, 150 μM was the highest concentration assayed.

Other aortic rings were depolarized with 60 mM KCl in the absence of calcium; when the baseline was reached, 1.5 mM $CaCl_2$ was added to evoke contraction, which was recorded for 20 min. This process was repeated until a reproducible response was obtained; then, the tissues were incubated with 5β-dihydrotestosterone at different concentrations (3, 10, 30, 100 or 150 μM, given separately) 10 min before the addition of $CaCl_2$ at 1.5 mM; this contraction induced by $CaCl_2$ was observed during 20 min, in the presence of the steroid. Subsequently, a $CaCl_2$-induced contraction was elicited again. After all calcium stimulus, the tissues were washout, three times, with depolarizing free-calcium solution. This protocol was used to test the potential calcium channel blocking properties of steroid.

The androgen effect was evaluated after 20 min and the concentration–response curves were obtained according to the method of Litchfield and Wilcoxon (1949).

In other series of experiments, the rings were pretreated with nifedipine 90 nM (dissolved in 0.1% ethanol), a single concentration of 5β-dihydrotestosterone (30 μM) or nifedipine (90 nM) + 5β-dihydrotestosterone (30 μM). The effects produced were noted 10 min before or 10 min after the noradrenaline- or KCl-induced contraction, recorded during 20 min. Their effects on the vasoconstrictor responses were compared with the contractions elicited in those rings without treatment.

The response elicited by the dihydropiridine-calcium channel activator (Bay K 8644, dissolved in 0.1% ethanol) was evaluated at different concentrations (1, 10 and 100 μM) 10 min before the contractions to noradrenaline or KCl were induced. The E_{max} of Bay K 8644 was established at 10 μM; this concentration was tested 10 min before and 10 min after the addition of 5β-dihydrotestosterone (30 μM) on the noradrenaline- or KCl-induced contraction. The effect of the steroid was also recorded during 10 min, compared with its effect without Bay K 8644 and estimated in terms of percent of diminution of the relaxing effect of 5β-dihydrotestosterone and percent of recovery of the contraction induced by each vasoconstrictor agent in the presence of steroid.

In another assay, different concentrations (1, 10 and 100 μM) of the $GABA_A$ agonist, muscimol (dissolved in 0.1% ethanol) or antagonists: picrotoxin (dissolved in 0.1% ethanol) or bicuculline (dissolved in 0.1 N KCl for dilution into the Krebs solution just before use) were applied, independently, 5 min after the noradrenaline contraction

was induced, observing their responses for 20 min. Each of the $GABA_A$ receptor antagonists, at the highest concentration (100 μM), were also tested 10 min before the addition of 30 μM 5β-dihydrotestosterone using this protocol. The steroid effect was observed for 10 min and compared in the absence of the $GABA_A$ receptor antagonists. The final concentration of vehicles: ethanol (0.2%) or HCl (0.1 N) plus ethanol (0.1%), in the bath did not modify the tonic contraction induced by noradrenaline.

Some additional experiments were carried out to analyze the possible interaction of the steroid with the β-adrenoceptors. For this purpose, different concentrations of isoproterenol (a non-selective β-adrenoceptor agonist) were applied 10 min after the contraction induced by 0.3 μM noradrenaline had been elicited, and its relaxing effect was evaluated for 10 min. Previously, we observed that the relaxation induced by 100 μM of isoproterenol on noradrenaline contraction was completely blocked when the tissues were incubated 5 min before with 20 μM propranolol (a non-selective β-adrenoceptor antagonist). Following the same protocol, the effect of 5β-dihydrotestosterone (30 μM) was observed with 20 μM propranolol incubation. Isoproterenol and propranolol were dissolved in distilled water.

After all of the above experiments had been concluded, the tissues were washed out, and a last contraction elicited by each vasoconstrictor agent was induced to observe the tissue recovery. In all tension studies, only one blood vessel ring was used from each rat; therefore, the *n* values refer to the number of animals from which the blood vessel rings were studied. Only one agonist or antagonist was tested in each ring.

2.3. Drugs

Apart from Bay K 8644, nifedipine (both from INC Pharmaceuticals, Costa Mesa, CA, USA) and the vehicle of steroids (ethanol from Merck-Mexico), the remaining compounds used in the present study were all purchased from Sigma (St. Louis, MO, USA), and included: 17β-hydroxy-5β-androstan-3-one (5β-dihydrotestosterone, 5β-DHT); 1,3,5(10)-estratrien-3,17β-diol (17β-estradiol, 17β-E_2); muscimol; bicuculline methiodide; picrotoxin; acetylcholine; noradrenaline; isoproterenol and propranolol.

2.4. Data presentation and statistical analysis

All data in the text and figures are presented as means ($n = 6$) ± S.E.M. Changes in tension are shown as percentage of the inhibition of the contraction induced by noradrenaline or KCl. Statistical significance was assessed by Student's *t*-test for values in two samples and Dunnett's test for values in multiple samples. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of 5β-dihydrotestosterone on noradrenaline- or KCl-induced contraction

Addition of 0.3 μM noradrenaline or 60 mM KCl to the aortic thoracic rings without endothelium caused a tonic contraction, and increasing concentrations of 5β-dihydrotestosterone induced an immediate concentration-dependent vasorelaxation of these contractions; in both cases, these tonic contractions declined with time. As shown in Fig. 1, 5β-dihydrotestosterone was more effective to relax the KCl-induced contraction than that induced by noradrenaline. The relaxing potency of 5β-dihydrotestosterone was higher than that induced by 17β-estradiol on the vasoconstrictor responses; thus, their IC_{50} values showed that 5β-dihydrotestosterone is 197.74 and 1.89 fold more potent to relax noradrenaline- and KCl-induced contrac-

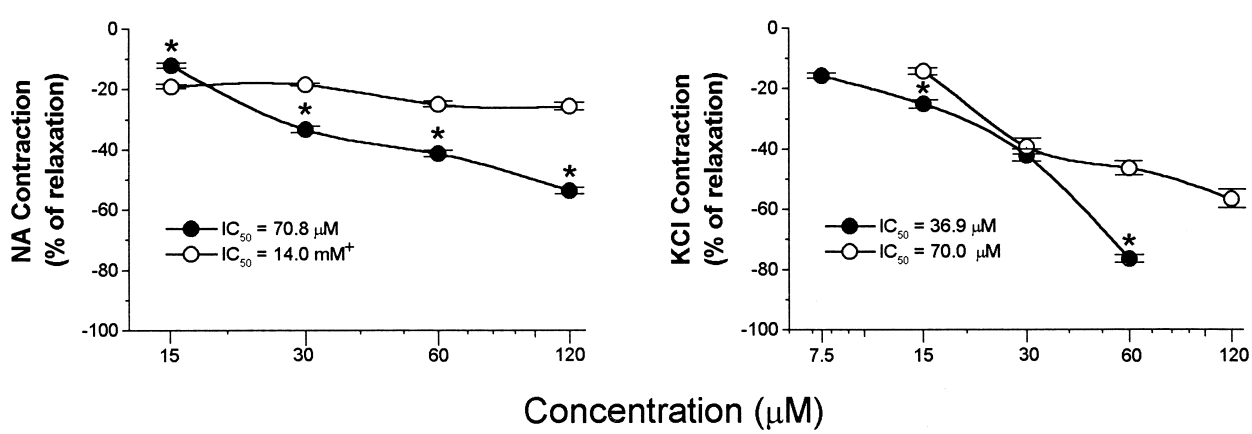

Fig. 1. Concentration–response curve of 5β-dihydrotestosterone (●) and 17β-estradiol (○) on the contraction induced by noradrenaline (NA 0.3 μM) or high potassium (KCl 60 mM) in isolated rat aorta. Each point represents the mean ($n = 6$) ± S.E.M. +Theoretical IC_{50} value; *$P < 0.0001$ 17β-estradiol vs. 5β-dihydrotestosterone.

tions, respectively. The stable tension produced by the vasoconstrictor agents without 5β-dihydrotestosterone in the same aortic rings was used as control for comparison. Ethanol added at 17.14 mM (0.1%, a concentration identical to those used as solvent for 5β-dihydrotestosterone) did not significantly affect these contractions, but the steroid effect was significantly different from the control with solvent ($P < 0.0001$). The development of the vasorelaxing effect in precontracted vessels started within a few seconds (~ 45 s) after addition of 5β-dihydrotestosterone; this effect was reversible upon washing out the hormone (traces A and B of Fig. 2).

3.2. Effect of 5β-dihydrotestosterone on calcium-induced contraction

The steroid vehicle (17.14 mM ethanol) did not affect the tonic contraction induced by 1.5 mM calcium. Interestingly, 5β-dihydrotestosterone induced a concentration-dependent blockade of the calcium contractions (trace C of

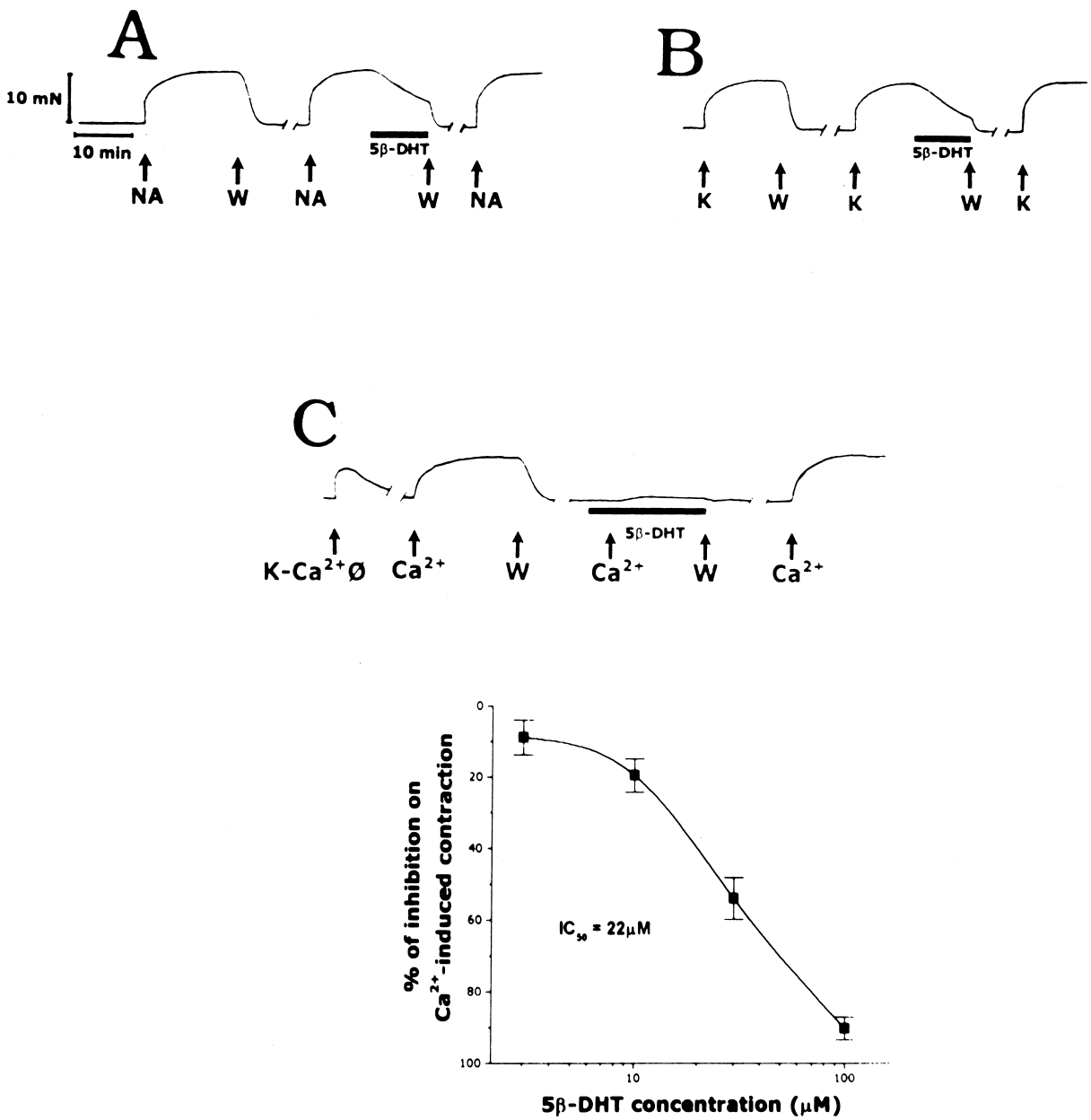

Fig. 2. Illustrates typical tracing in isolated rat aorta without endothelium. 5β-Dihydrotestosterone (5β-DHT 30 μM) relaxed the contraction induced by: (A) noradrenaline (NA, 0.3 μM) and (B) high potassium solution (K, 60 mM). (C) 5β-DHT also prevented the contraction induced by calcium (Ca^{2+} 1.5 mM) in depolarized tissue immersed in high potassium calcium-free media (K–Ca^{2+}∅); lower panel shown the concentration–response curve of this calcium antagonic effect induced by 5β-DHT. The short black line indicates the incubation time of steroid. Note the contraction recovery after washout (W), showing that the steroid effect was reversible.

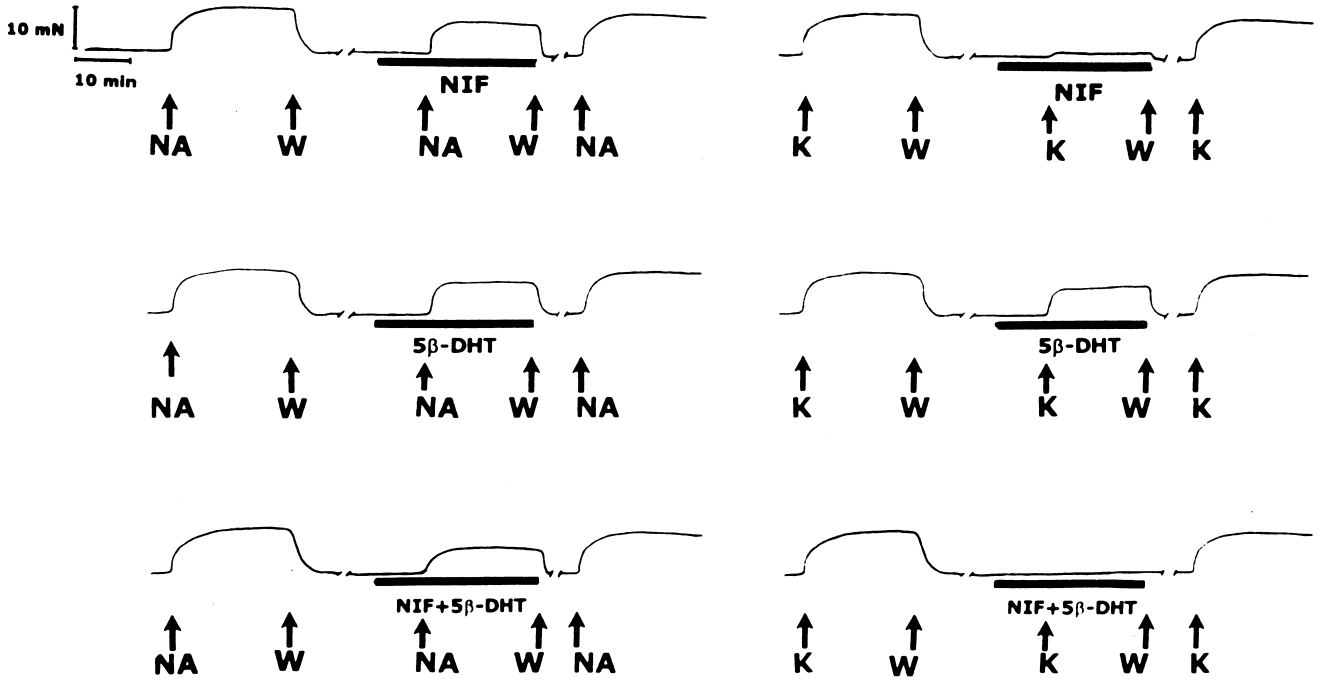

Fig. 3. Prevention induced by nifedipine (NIF 90 nM), 5β-dihydrotestosterone (5β-DHT 30 μM), or NIF + 5β-DHT on noradrenaline (NA 0.3 μM) or high potassium solution (K 60 mM) contractions in isolated rat aorta. The short black line indicates the incubation time of the different treatments. The contraction recovery is observed after washout (W).

Fig. 2, lower panel). Maximum blockade (100%) was achieved at 150 μM of 5β-dihydrotestosterone, and its IC_{50} value was 22 μM. The calcium antagonic effect induced by the hormone was reversible upon washing out the tissue (trace C of Fig. 2).

3.3. Pretreatment with nifedipine

For the sake of comparison, the blockade of contractions elicited by noradrenaline and KCl with 90 nM nifedipine was also studied; this dihydropyiridine-calcium channel inactivator displayed the highest potency in blocking the KCl-contraction, with 86.9 ± 1.5% of inhibition, and only 19.9 ± 1.7% of inhibition on the noradrenaline-contraction. However, the hormone showed a higher prevention than nifedipine on the noradrenaline-induced contraction ($P < 0.01$). It is important to emphasize that nifedipine did not reduce the 5β-dihydrotestosterone effect, but it was synergized (Fig. 3). Some experiments

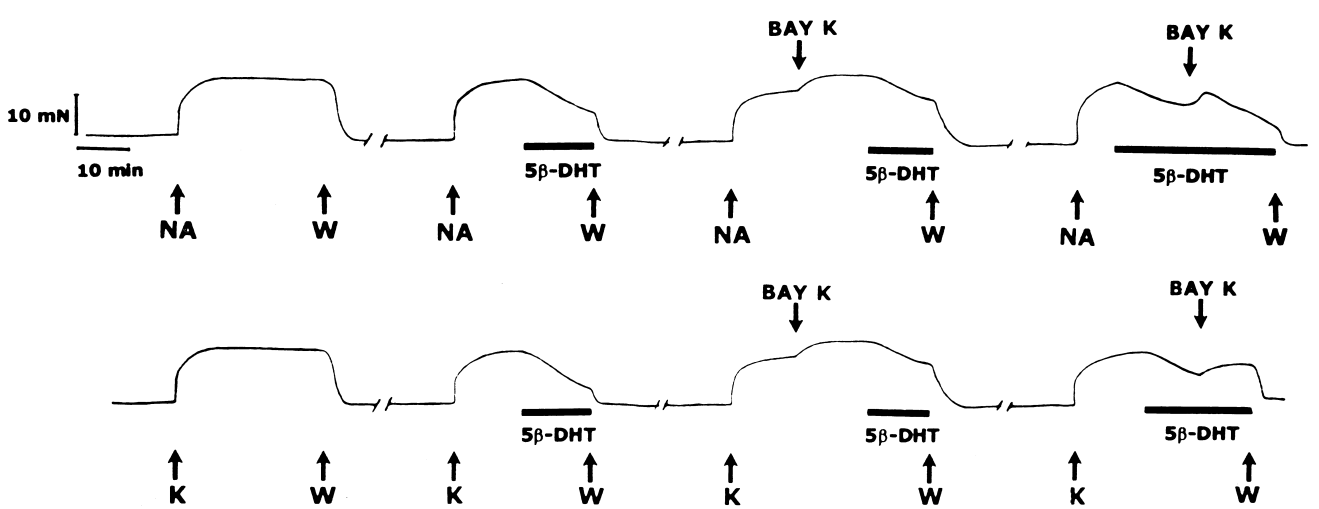

Fig. 4. The relaxing effect of 5β-dihydrotestosterone (5β-DHT 30 μM) on the contraction induced by noradrenaline (NA 0.3 μM) and high potassium (K 60 mM) was diminished or reverted by BAY K (dihydropiridine-calcium channel activator 10 μM) addition, in the isolated rat aorta. The short black line indicates the incubation time of steroid, after each treatment the tissues were washed out (W).

were performed adding nifedipine and/or 5β-dihydrotestosterone on the contraction tone of the vasoconstrictor responses; a synergism between both compounds was also observed (data not shown).

3.4. Effects of Bay K 8644 and steroid relaxing action

Bay K 8644 induced a small but concentration-dependent tonic contraction amounting to 16.8 ± 0.6% and 18.6 ± 0.8% ($n = 6$) of the 0.3 μM noradrenaline and 60 mM KCl, respectively. These were the E_{max} values obtained at 10 μM of the dihydropyiridine-calcium channel activator (Bay K 8644). This concentration was also used to observe that the vasodilating effect of 5β-dihydrotestosterone was significantly attenuated in the presence of Bay K 8644 ($P < 0.001$); the steroid effect was reverted after the addition of Bay K 8644 ($P < 0.001$) on both noradrenaline- and KCl-contraction. However, the relaxing effect of the steroid on noradrenaline-induced contraction was temporally reverted by the addition of Bay K 8644 (Fig. 4).

3.5. Effects of agonists and antagonists at $GABA_A$ receptors

Different concentrations of muscimol, bicuculline and picrotoxin did not modify the tone and amplitude of the contraction induced by noradrenaline (not shown). Furthermore, both bicuculline or picrotoxin did not block the vasorelaxing effect of steroid ($P > 0.05$) (see Fig. 5).

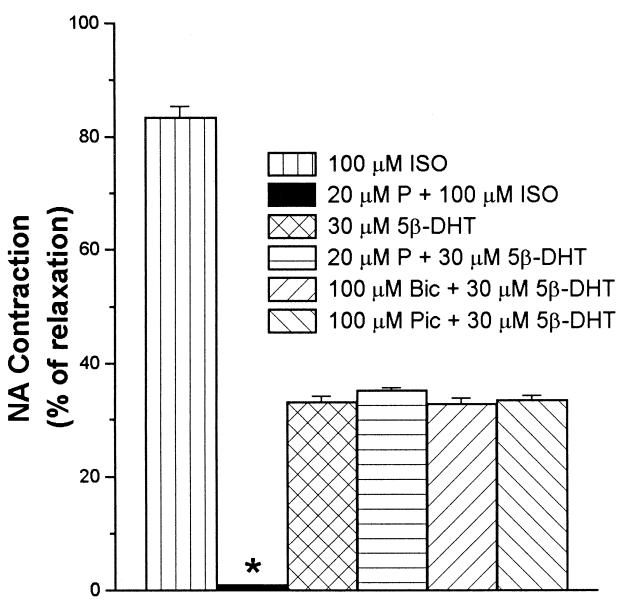

Fig. 5. Percent of relaxation of isoproterenol (ISO) and 5β-dihydrotestosterone (5β-DHT) with or without β-adrenoceptor antagonist propranolol (P), and both $GABA_A$ antagonists: bicuculline (Bic) or picrotoxin (Pic) on the tonic contraction induced by noradrenaline (NA 0.3 μM) in rat aorta. Each bar represents the mean ($n = 6$); vertical lines indicated ± S.E.M. The 5β-DHT effect was not significantly different in presence of the antagonists ($P > 0.05$, $^*P < 0.0001$).

3.6. Effect of compounds interacting with β-adrenoceptors

The vasorelaxing effect of 100 μM isoproterenol (a β-adrenoceptor agonist) on the noradrenaline-induced contraction was completely blocked by 20 μM propranolol (a β-adrenoceptor antagonist). In marked contrast, the vasorelaxing effect of 5β-dihydrotestosterone was not blocked by propranolol at 20 μM (Fig. 5).

4. Discussion

4.1. General

Several lines of evidence imply that ovarian steroids are vasodilator substances. In this respect, it has been shown that blood pressure decreases when spayed rats are treated with estradiol (Fischer and Swain, 1977) or during pregnancy (Elkayam, 1992), when progesterone and estrogen levels are high. In addition, high estrogen levels increase the uterine blood flow during estrus (Stice et al., 1987a,b). Consistent with these findings, 17β-estradiol has been shown to possess a hypotensive action (Magness and Rosenfeld, 1989; Vargas et al., 1995).

The hypotensive action of some steroids may be directly related with the vasodilating effect found for these compounds. Indeed, estrogens can produce vasodilatation in several vascular beds including uterine, umbilical and skin blood vessels, portal vein and atherosclerotic coronary arteries (McCalden, 1975; Silva de Sa and Meirelles, 1977; Raddino et al., 1986; Magness and Rosenfeld, 1989; Vargas et al., 1989; Williams et al., 1990; Shan et al., 1994). Furthermore, we have recently demonstrated that 5-reduced androgens and progestins can induce a non-genomically mediated vasodilating effect on endothelium-denuded rat aortic rings, with 5β-dihydrotestosterone showing the highest vasorelaxant potency (Perusquía et al., 1996).

Considering the above findings, it is reasonable to assume, as previously implied for female sex steroids, that male sex steroids might also play an important physiological role providing ‘protection’ from cardiovascular pathologies during sexual age. In an attempt to approach this conception, we considered it important to analyze, in principle, the possible mechanisms underlying the vasorelaxant action of 5β-dihydrotestosterone. Our results clearly show that 5β-dihydrotestosterone is a vasodilator substance, supporting the view that the cardiovascular system might be the target for male sex steroids.

The steroid response latency has been used as an indicator of genomic versus nongenomic mechanisms of action (McEwen, 1991). Therefore, the rapid (< 1 min) and reversible relaxing effect of 5β-dihydrotestosterone could be explained as a nongenomic (membrane) action, which is not mediated by the classical intracellular steroid receptors. Nevertheless, the cellular or molecular basis of steroid

action on vascular smooth muscle has not yet been clearly defined.

4.2. Is the nongenomic vasodilator action of 5β-dihydrotestosterone related to an interaction with $GABA_A$ receptors

In view that some neurosteroids produce their depressive effects on the brain via an interaction with $GABA_A$ receptors (Harrison and Simmonds, 1984; Majewska et al., 1986; Gee et al., 1987, 1988; Harrison et al., 1987; Morrow et al., 1987, 1990; Peters et al., 1988; Turner et al., 1989; Lan et al., 1990), we decided to investigate, in the first instance, if such a mechanism is involved in the vasodilator action of 5β-dihydrotestosterone. Nevertheless, our results do not support an interaction of 5β-dihydrotestosterone with $GABA_A$ receptors since: (i) muscimol, a $GABA_A$ receptor agonist, failed to produce any response at all the concentrations tested; and (ii) bicuculline and picrotoxin, which are potent $GABA_A$ receptor antagonists, failed to block the vasodilating effect of 5β-dihydrotestosterone. These evidences are also suggesting that the $GABA_A$ receptors are not present in the extracranial vessels. Consistent with these findings, similar (and even higher) concentrations of bicuculline and picrotoxin have also failed to block the relaxation induced by 5β-dihydrotestosterone and other steroids in the rat uterus (Perusquía and Villalón, 1996) and in guinea-pig trachea (Perusquía et al., 1997).

4.3. Is the nongenomic vasodilator action of 5β-dihydrotestosterone related to an interaction with β-adrenoceptors

Although our study has excluded an interaction of 5β-dihydrotestosterone with $GABA_A$ receptors (see above), our experimental conditions cannot categorically exclude a possible interaction of 5β-dihydrotestosterone with vascular β-adrenoceptors which, upon activation, increase cyclic AMP levels inhibiting calcium influx, and thereby act to produce vasorelaxation (see Sperelakis, 1990; Hoffman and Lefkowitz, 1996). Certainly, there is no line of pharmacological evidence showing a direct interaction of 5β-dihydrotestosterone (or any other steroid) with β-adrenoceptors in vascular smooth muscle, but a potential action of 5β-dihydrotestosterone on vascular sympathetic nerves displacing catecholamines (tyramine-like action) cannot be ruled out, as previously shown for other substances, including 5-hydroxytryptamine (see Saxena and Villalón, 1990). If this were the case, the catecholamines displaced by 5β-dihydrotestosterone may have interacted with vascular α- and β-adrenoceptors; under circumstances favoring a greater interaction with vascular β-adrenoceptors, the resulting effect would be vasorelaxation and this effect, therefore, should have been amenable to blockade by β-adrenoceptor antagonists, including propranolol (see Hoffman and Lefkowitz, 1996). However, our study excludes this possibility, as propranolol did not modify the vasorelaxing effect of 5β-dihydrotestosterone (Fig. 5). This concentration of propranolol (20 μM) was high enough to antagonize vasodilator β-adrenoceptors, as the vasorelaxant effect produced by the β-adrenoceptor agonist, isoproterenol (Hoffman and Lefkowitz, 1996) was abolished by the same concentration of propranolol.

4.4. Does the nongenomic vasodilator action of 5β-dihydrotestosterone involve an interaction with calcium channels

Since the involvement of $GABA_A$ receptors and/or β-adrenoceptors seems improbable, the possibility has to be discussed finally that the vasorelaxing effects of 5β-dihydrotestosterone involve an interaction with calcium channels. Regarding the vasodilating potency of androgens, it is important to point out that 5β-dihydrotestosterone was more potent than 17β-estradiol to induce relaxation. Thus, it is tempting to suggest that the androgens could be better protectors than the estrogens against cardiovascular diseases; admittedly, much further work will be needed to document and evaluate this possibility. In this respect, our preliminary results show that the vasorelaxation induced by 5β-dihydrotestosterone was more prominent on the contraction induced by KCl than on that induced by noradrenaline.

The simplest interpretation of the above findings suggests that 5β-dihydrotestosterone-induced vasorelaxation may involve a diminution on the intracellular concentration of calcium in the vascular smooth muscle cells of rat aorta. In addition, our findings showed that the tonic component in both noradrenaline and KCl contractions were highly sensitive to the relaxing effect of steroid, suggesting that the steroid effect is a consequence of the calcium entry blockade, due to the fact that, in other arteries, it has been suggested that the initial rapid and the ensuing tonic component are due to intracellular calcium release and calcium influx, respectively (Bolton, 1979; Van Breemen et al., 1982; Skärby et al., 1984). In this context, it is known that KCl depolarizes the membrane and opens voltage-operated channels (VOCs), resulting in calcium entry, whilst noradrenaline is able to open receptor-operated channels (ROCs) to induce calcium influx in aorta, without producing membrane depolarization (Cauvin et al., 1985). Therefore, the high potency of 5β-dihydrotestosterone to elicit relaxation on KCl-induced contraction might be distinguishing the source and kind of calcium gates involved in the relaxing action of the steroid.

Consequently, the potent relaxation induced by 5β-dihydrotestosterone in aortic rings precontracted by KCl is implying a reduction of extracellular calcium influx by inactivating of VOCs. Consistent with this view: (i) 5β-dihydrotestosterone was able to abolish the calcium-induced contraction in depolarized tissues (see Fig. 2C), suggesting

that the steroid could be an 'endogenous calcium channel antagonist'; (ii) 5β-dihydrotestosterone elicited minor relaxation in the contractions evoked by Bay K 8644. Likewise, the vasorelaxant effect of 5β-dihydrotestosterone in aortic rings precontracted with KCl or noradrenaline was markedly reverted by Bay K 8644, implying a competitive antagonism of 5β-dihydrotestosterone, as reported for the dihydropyridine calcium antagonists in smooth muscle (Towart and Schramm, 1984; Spedding, 1985).

However, the loss of tone contraction elicited by 5β-dihydrotestosterone was temporarily recovered by Bay K 8644 in the noradrenaline-induced contraction, but maintained in KCl-induced contraction; this may imply that (i) noradrenaline induces increases in intracellular calcium by activating ROCs or releasing calcium from intracellular storage sites; and (ii) Bay K 8644 promotes calcium influx with dihydropyridine receptors on the VOCs. On this basis, one would expect that the vasorelaxation elicited by 5β-dihydrotestosterone is a consequence of calcium entry the blockade via VOCs; nevertheless, since 5β-dihydrotestosterone was also able to induce vasorelaxation in rings precontracted with noradrenaline, an agonist of receptor-operated calcium channels (Cauvin et al., 1985), our results indicate that 5β-dihydrotestosterone has similar relaxant effects on the contractions induced by activation of both receptor- and voltage-operated calcium channels.

Indeed, both noradrenaline- and KCl-induced contractions were prevented, to a *similar* extent, by 5β-dihydrotestosterone preincubation, in contrast to that by the dihydropyridine calcium channel antagonist, nifedipine. This effect, undoubtedly, is associated with VOCs to inhibit calcium entry, which was more potent to antagonize the KCl-induced contraction. This correlates well with previous studies showing that the contractions induced by noradrenaline are more dependent on intracellular calcium release (Casteels et al., 1977) and, consequently, less inhibited by calcium antagonists (Van Breemen et al., 1982). From these findings, one could suggest that nifedipine and 5β-dihydrotestosterone possess a different site of action on VOCs; however, we cannot rule out that 5β-dihydrotestosterone is also inactivating the ROCs because the androgen was more effective than nifedipine to prevent the noradrenaline-induced contraction. In support of this view, nifedipine plus 5β-dihydrotestosterone summarized the prevention as well as the relaxation in both vasoconstrictor responses, although this additive effect inhibited the contractions induced by KCl significantly more than those induced by noradrenaline (see Fig. 3). Likewise, other studies have shown that high estrogen levels may increase uterine blood flow during estrus by blocking vascular smooth muscle voltage-sensitive calcium channels in pig uterine arteries (Stice et al., 1987a,b), the estrogens may also reduce intracellular calcium via a potential-operated calcium channel mechanism in gilts uterine vasculature (Ford et al., 1984) and in rabbit isolated coronary arterial preparations (Jiang et al., 1991); furthermore, the inhibitory effect of estradiol on contractile responses to pheylephrine and prostaglandin $F_{2\alpha}$ in pig left anterior descending coronary artery and rat thoracic aorta has also been associated with prevention of calcium inflow (Vargas et al., 1989). Additionally, a direct inhibitory effect of 17β-estradiol on voltage-dependent calcium currents has been demonstrated in A7r5 vascular smooth muscle cell line (Zhang et al., 1994), and in coronary and tail artery myocytes (Shan et al., 1994; White et al., 1995). On the other hand, it has also been reported that the relaxation induced by progestins and androgens in other kind of smooth muscles may reduce intracellular calcium in uterine and airway cells by blocking the calcium influx through the VOCs and ROCs (Perusquía et al., 1990, 1991a,b, 1997; Perusquía and Campos, 1991; Perusquía and Kubli-Garfias, 1992; Perusquía and Villalón, 1996).

In closing, the above calcium-antagonistic properties could be one of the mechanisms involved in 5β-dihydrotestosterone-induced endothelium-independent relaxation in rat aortic rings. Although these results, admittedly, do not allow any extrapolation with the systemic vasculature, it is tempting to suggest that male sex hormones may play a 'protective' vascular role which might be correlated with the beneficial effects reported for the androgenic hormone, dehydroepiandrosterone (DHEA; a metabolic intermediate in the pathway for the synthesis of androgens and estrogens). This hormone is associated with increased longevity and decreased cardiovascular disease (Regelson et al., 1988, 1990), which could be awarded to products from its metabolism, such as androgens and/or estrogens.

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