available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Sexual Medicine

Hydrogen Sulphide Is Involved in Testosterone Vascular Effect

Mariarosaria Bucci[a], *Vincenzo Mirone*[b], *Annarita Di Lorenzo*[a], *Valentina Vellecco*[a], *Fiorentina Roviezzo*[a], *Vincenzo Brancaleone*[a], *Imbimbo Ciro*[b], *Giuseppe Cirino*[a,b,*]

[a] *Dipartimento di Farmacologia Sperimentale, Universita' di Napoli Federico II, Napoli, Italia*
[b] *Centro Interdipartimentale di Ricerca Preclinica e Clinica di Medicina Sessuale (CIRMS), Universita' di Napoli Federico II, Napoli, Italia*

Article info

Article history:
Accepted May 7, 2008
Published online ahead of print on May 22, 2008

Keywords:
Testosterone
Hydrogen sulphide
L-cysteine
Propargylglycine
β-cyano-L-alanine
Cystathionine γ-lyase
Vascular reactivity

Abstract

Background: Testosterone (T) induces a rapid relaxation in vascular tissues of different species due to a nongenomic effect of this steroid on vessels. Different mechanisms have been proposed to explain T-induced vasodilatation but the effective mechanism(s) and the mediators involved are still a matter of debate.
Objectives: We have evaluated if H_2S pathway is involved in T vascular effects.
Design and setting: Male Wistar rats were sacrificed and thoracic aorta was rapidly dissected and cleaned from fat and connective tissue. Rings of 2–3 mm length were cut and placed in organ baths filled with oxygenated Krebs solution at 37 °C and mounted to isometric force transducers. H_2S determination was performed on thoracic aortic rings incubated with T or vehicle and in presence of inhibitors. H2S concentration was calculated against a calibration curve of NaHS (3–250 μM). Results were expressed as nmoles/mg protein.
Measurements: Vascular reactivity was evaluated by using isometric transducers. H_2S determination was performed by using a cystathionine β-synthetase (CBS) and cystathionine γ lyase (CSE) activity assay. CSE and CBS protein levels were assessed by Western blot analysis. Statistical analysis was performed by using two-way ANOVA and unpaired Student's t-test where appropriate.
Results: T significantly increased conversion of L-cysteine to H_2S. This effect was significantly reduced by PGG and BCA, two specific inhibitors of CSE. T (10 nM–10 μM) induced a concentration-dependent vasodilatation of rat aortic rings in vitro that was significantly and concentration-dependent inhibited by PGG, BCA, and glybenclamide. Incubation of aorta with T up to 1 h did not change CBS/CSE expression, suggesting that T modulates enzymatic activity.
Conclusions: Here we demonstrate that T vasodilator effect involves H_2S, a novel gaseous mediator. T modulates H_2S levels by increasing the enzymatic conversion of L-cysteine to H_2S.

Abbreviations: T, testosterone; CBS, cystathionine β-synthetase; CSE, cystathionine γ-lyase; TEA, tetraethylammonium; PEG, polyethylene glycol; PGG, propargylglycine; BCA, β-cyano-L-alanine; PE, phenylephrine; Ach, acetylcholine; DMSO, dimethyl sulfoxide; DPD, N,N-dimethylphenylenediamine sulphate; PP, pyridoxal-5'-phosphate hydrate; TCA, trichloroacetic acid; Gly, glybenclamide.

* Corresponding author. Dipartimento di Farmacologia Sperimentale, via Domenico Montesano 49, 80131 Napoli, Italy. Tel. +39 081 678442; Fax: +39 081 678403.
E-mail address: cirino@unina.it (G. Cirino).

0302-2838/$ – see back matter doi:10.1016/j.eururo.2008.05.014

1. Introduction

In the last two decades, growing epidemiologic, clinical, and experimental evidence have shown a marked sexual dimorphism in cardiovascular function and in the pathogenesis of cardiovascular disease. Numerous studies have correlated reduced circulating levels of testosterone to severe coronary artery disease (CAD) [1], atherosclerotic risk [2,3], and other pathologies, including hypertension, diabetes and insulinaemia [4], implicating a possible beneficial effect of testosterone on the cardiovascular system. In addition, it has been reported that chronic testosterone treatment reduces serum levels of IL-1 beta, TNF alpha, and increases the levels of the anti-inflammatory cytokine IL-10 [5,6], conferring to this hormone long lasting cardiovascular benefits. Several studies have shown that acute administration of testosterone induces a rapid relaxation in vascular tissues of different species including humans [8–11], suggesting a nongenomic effect of this hormone on vascular reactivity. Different mechanisms have been proposed to explain testosterone-induced vasodilatation [7,8,12] but it remains a matter of debate which is the effective mechanism(s) and which are the mediators involved of the testosterone-induced vasorelaxation.

Hydrogen sulphide (H_2S) is an endogenous gas produced in mammals from L-cysteine by two different enzymes: cystathionine β-synthetase (CBS), predominantly present in central nervous system (CNS), and cystathionine γ-lyase (CSE), predominantly localised in the cardiovascular network [13–15]. Recently, H_2S has been shown to act as gaseous modulator on rat vascular system both in vivo [16] and in vitro [17]. Some studies have suggested that testosterone-induced vasodilatation may involve activation of vascular smooth muscle potassium channels [18,19]. The finding that activation of potassium channels is involved in testosterone-induced vasodilatation led us to hypothesize a possible involvement of H_2S as a mediator involved, since drugs that block K_{ATP} channels, such as glibenclamide, have been shown to block the relaxant effect caused by exogenous H_2S [20,21]. Here, we have evaluated the possible involvement of H_2S in the vasorelaxant effect of testosterone by using rat isolated aorta.

2. Materials and methods

2.1. Materials

Acetylcholine (ACh), L-phenylephrine (PE), tetraethylammonium (TEA), testosterone (T), polyethylene glycol 400 (PEG), dimethyl sulfoxide (DMSO), propargylglycine (PGG), β-cyano-L-alanine (BCA), N,N-dimethylphenylenediamine sulphate (DPD), pyridoxal-5′-phosphate hydrate (PP), iron chloride ($FeCl_3$), zinc acetate (ZnAc), NaHS, L-cysteine, and glybenclamide (gly) were purchased from Sigma Chemical Co (Milan, Italy). Trichloroacetic acid (TCA) was purchased from Carlo Erba Reagents (Milan, Italy). Dulbecco's modified Eagle medium (DMEM), L-glutamine, penicillin, and streptomycin were purchased from Hy-Clone (Road Logan, UT, USA). Testosterone was dissolved in PEG, glybenclamide in DMSO, TEA and PGG in dH_2O. The volume added to the organ bath for each compound was 2.5 μl, except for TEA, which was 25 μl.

2.2. Tissue preparation

Male Wistar rats (Charles River, 250–300 g) were sacrificed and thoracic aorta was rapidly dissected and cleaned from fat and connective tissue. Rings of 2–3 mm length were cut and placed in organ baths (2.5 ml) filled with oxygenated (95%O_2–5%CO_2) Krebs solution at 37 °C and mounted to isometric force transducers (type 7006, Ugo Basile, Comerio, Italy) and connected to a Graphtec linearcorder (WR3310, Japan). The composition of the Krebs solution was as follows: (mM): NaCl 118, KCl 4.7, $MgCl_2$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, and glucose 10.1. Rings were initially stretched until a resting tension of 0.5 g was reached and allowed to equilibrate for at least 30 min, during which tension was adjusted, when necessary, to 0.5 g and bathing solution was periodically changed. In a preliminary study, a resting tension of 0.5 g was found to develop the optimal tension to stimulation with contracting agents. The studies were carried out in accordance with the Declaration of Helsinki of the European Community guidelines for the use of experimental animals.

2.3. Experimental protocol

In each experiment, rings were first challenged with PE (1 μM) until the responses were reproducible. In order to verify the integrity of the endothelium, Ach cumulative concentration-response curve (10 nM–30 μM) was performed on PE-precontracted rings. The rings were then washed and contracted with PE (1 μM) and, once a plateau was reached, a cumulative concentration-response curve of testosterone (10 nM–10 μM) performed. In another set of experiments, rings were contracted with KCl (60 mM) and a cumulative concentration-response curve of testosterone (10 nM–10 μM) performed.

2.4. Drug treatments

The optimal incubation time of the drug treatments and concentration were selected from literature as specified for each protocol below reported. The experimental protocols were:

(1) TEA (10 mM) [22] and Glybenclamide (10, 50 μM) [20] were added in the organ baths. After 60 and 30 min, respectively, the rings were contracted with PE (1 μM) and a cumulative concentration-response curve of T was performed.
(2) PGG and BCA [23,24] (1, 3, 10 mM) were added in the organ baths, after 15 and 60 min respectively, rings were contracted with PE (1 μM) and cumulative concentration response-curve of T was performed.
(3) Rings were contracted with KCl 60 mM, once plateau was reached cumulative concentration-response curve of T was performed.

Drug addition and incubation time selected did not alter the contraction induced by PE (data not shown).

2.5. CSE/CBS activity assay

H_2S determination was performed according to Stipanuk and Beck [25] with modifications. Briefly, the thoracic aorta was dissected, placed in sterile phosphate buffer solution, and cleaned of fat and connective tissue. Rings, of the same size as described above, were cut and placed in 24 plate with 990 μl Krebs solution for 30 min at 37 °C in an incubator (Mod. BB6220, Heraeus Instruments, Germany) with humidified air (5% CO_2/95% O_2). After this time, T (10 μM) or vehicle (PEG; 10 μl) was added and the rings were incubated for 5, 15, 30, and 60 min. In another set of experiments, aortic rings were exposed to PGG or BCA (10 mM) for 15 and 60 min respectively, then testosterone (10 μM) or vehicle were added for 30 and 60 min. At the end of the incubation time, the aortic

rings were homogenized in a lysis buffer (potassium phosphate buffer 100 mM pH = 7.4, sodium orthovanadate 10 mM and protease inhibitor) and the protein concentration was determined using Bradford assay (Bio-Rad Laboratories, Milan, Italy). The homogenates were added in a reaction mixture (total volume 500 μl) containing piridoxal-5′-phosphate (2 mM, 20 μl), L-Cysteine (10 mM, 20 μl), and saline (30 μl). The reaction was performed in parafilmed eppendorf tubes and initiated by transferring tubes from ice to a water bath at 37 °C. After incubation of 30 min, ZnAc (1%, 250 μl) was added to trap evolved H_2S followed by TCA (10%, 250 μl). Subsequently, DPD (20 μM, 133 μl) in 7.2M HCl and $FeCl_3$ (30 μM, 133 μl) in 1.2M HCl were added. After 20 min, absorbance values were measured at a wavelength of 650 nm. All samples were assayed in duplicate and H_2S concentration was calculated against a calibration curve of NaHS (3.12–250 μM). Results were expressed as nmoles/mg of protein.

2.6. Western blotting

The thoracic aorta was dissected, placed in sterile phosphate buffer solution, and cleaned of fat and connective tissue. Rings, of the same size as described above, were cut and placed in 24 plate with 990 μl DMEM solution (containing penicillin 100 U ml^{-1}, streptomycin 100 μg ml^{-1}, and L-glutamine 2 mM) for 30 min at 37 °C in an incubator (Mod. BB6220, Heraeus Instruments, Germany) with humidified air (5% CO_2/95% O_2). After this time, T (10 μM) or vehicle (PEG; 10 μl) was added and the rings were incubated for 15, 30 min, and 1 h. At the end of the incubation time, the aortic rings were homogenized on ice in the following lysis buffer: Tris-HCl 50 mM pH 7.4, NP-40 1%, Na-deoxycholate 0.25%, NaCl 150 mM, EDTA 1 mM, PMSF 1 mM, Aprotinin, leupeptin, 1 μg/ml each, Na_3VO_4 1 mM, NaF 1, and mM. After centrifugation at 12,000 rpm for 15 min, protein concentration was determined by Bradford assay using BSA as standard (Bio-Rad Laboratories, Milan, Italy). 30 μg of the denatured proteins were separated on 10% sodium dodecyl sulphate polyacrylamide gel and transferred onto a PVDF membrane (Millipore). Membranes were blocked by incubation in PBS containing 0.1%v/v Tween-20 and 5% nonfat dry milk for 1 h at room temperature, and then incubated with mouse anti-CBS polyclonal antibody (1:1000, Novus Biological) and mouse anti-CSE monoclonal antibody (1:500, Novus Biological) overnight at 4 °C. The membranes were washed extensively in PBS containing 0.1% v/v Tween-20 and then incubated with the secondary antibody (1:5000) conjugated with horseradish peroxidase, antimouse IgG for 2 h at 4 °C. The filters were then washed and the immoreactive bands were visualised using the enhanced chemiluminescence substrate (ECL, Amersham Pharmacia Biotech).

2.7. Statistical analysis

All data were expressed as mean ± SE mean. Statistical analysis was performed by using two-way ANOVA and unpaired Student t-test where appropriate. Differences were considered statistically significant when p was less than 0.05.

3. Results

In order to evaluate if testosterone could modulate H_2S production, we measured its effect on aorta following incubation with the vehicle or L-cysteine. Incubation of aortic rings with vehicle, eg, the basal levels of H_2S production, were unaffected by testosterone exposure, while the conversion of L-cysteine in H_2S was enhanced in tissues exposed to testosterone (Fig. 1). Indeed, incubation of aortic rings with testosterone at the concentration of 10 μM for 5, 15, 30, or 60 min significantly increased H_2S production after 30 and 60 min (Fig. 1). Meanwhile, the incubation for 5 and 15 min did not modify the production of H_2S (Fig. 1). Propargylglycine (PGG) and β-cyano-L-alanine (BCA), two inhibitors of CSE, significantly reduced T-induced H_2S production (Fig. 2), supporting the hypothesis that testosterone positively modulates L-cysteine-induced H_2S production through a direct action on CSE. To further address the involvement of H_2S in the testosterone-induced vasorelaxation, we performed an *in vitro* functional study using BCA and PGG in order to perform a pharmacologic modulation. Both PGG (Fig. 3b) and BCA (Fig. 3c) inhibited testosterone-induced vasorelaxation and this effect was concentration-dependent. These data confirmed that testosterone modulates H_2S release through CSE. Following this latter finding, we performed a Western blot analysis to evaluate if there was a change in protein expression of either CBS or CSE. The lack of change in protein expression (Fig. 4), within the time frame considered in this study, suggests that testosterone augmented H_2S synthesis by increasing enzyme activity rather

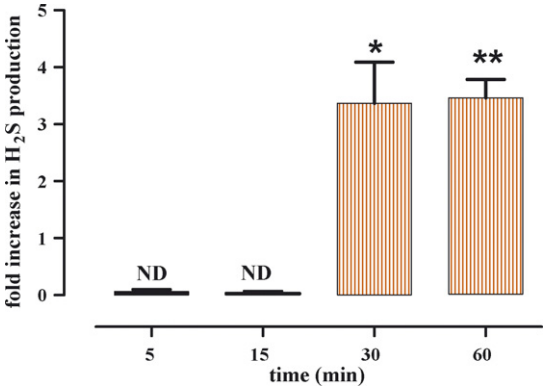

Fig. 1 – Time course of L-cysteine-stimulated H_2S production in rat aorta following incubation with T (10 μM) for 5, 15, 30, and 60 min. Control value (vehicle) of H_2S production was 153 ± 23 nmoles/mg protein. $n = 4$, * = $p < 0.05$, ** = $p < 0.01$ vs vehicle. Data are expressed as fold increase compared to vehicle.

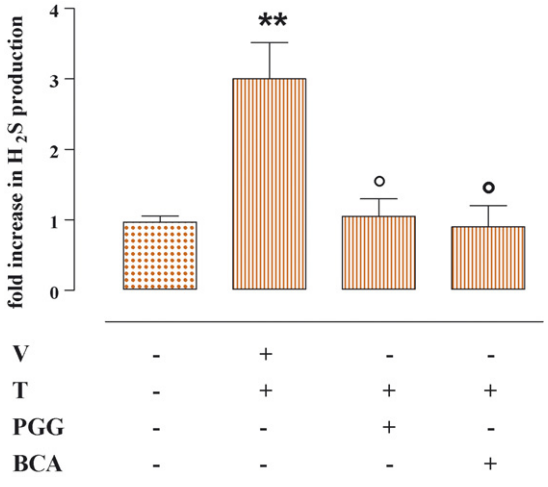

Fig. 2 – PGG and BCA (10 mM) inhibited testosterone-induced H_2S biosynthesis in rat aorta. V = vehicle (dH_2O), T = testosterone, PGG and BCA. ** = $p < 0.01$ vs C, ° = $p < 0.05$ vs V. Data are expressed as fold increase of H_2S production $n = 4$.

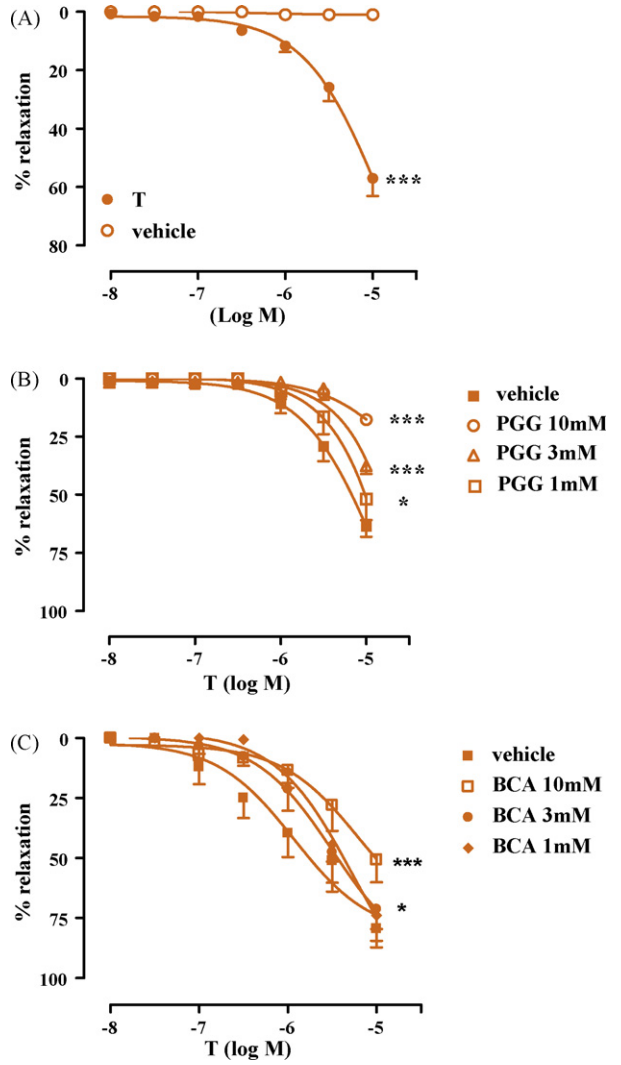

Fig. 3 – Cumulative concentration-response curve of T on rat aortic rings. *** = $p < 0.001$, $n = 7$ (panel A). Incubation with PGG (panel B), or BCA (panel C), at different concentrations, of rat aorta rings inhibited T-induced vasodilatation. ***$p < 0.001$, *$p < 0.05$, $n = 6$ for each set of experiments.

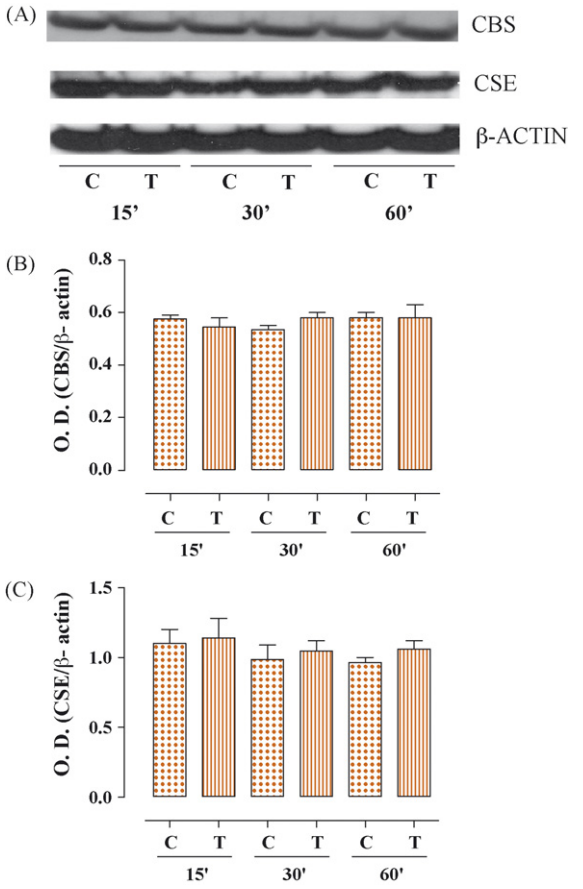

Fig. 4 – Testosterone incubation of rat aorta did not change CBS or CSE expression. Panel A shows a representative Western blot for CBS and CSE. Panels B and C show Western blot densitometric analysis densitometry for CBS and CSE, respectively ($n = 3$ experiments).

than expression. Next, we have confirmed that in our experimental conditions potassium channels are involved in testosterone-induced vasorelaxation (Fig. 5). The testosterone cumulative concentration response curve was significantly inhibited when rings were contracted with KCl (Fig. 5a) or in presence of TEA (Fig. 5b) as opposite to PE (Fig. 5a) implying the involvement of potassium channels. Incubation of aortic rings with Gly, a specific inhibitor of K_{ATP} channels, concentration-dependently inhibited testosterone-induced vasorelaxation, confirming that T exerts its effect, in our experimental conditions, mainly through K_{ATP} channels (Fig. 5c).

4. Discussion

Hydrogen sulphide is a gaseous mediator that has been shown to play a role in the cardiovascular system. It has been hypothesised that one of the mechanisms involved in its action is activation of K_{ATP} channels [26]. Since K_{ATP} channels have been shown to be involved in testosterone effects [20,21] we evaluated if H_2S is involved downstream in testosterone signalling. We first verified the involvement of H_2S in testosterone-induced vasodilatation by operating a pharmacologic modulation. Incubation of aorta rings with PGG or BCA, two different CSE inhibitors of L-cys/H_2S pathway, led to a significant and concentration-dependent inhibition of testosterone-induced vasodilatation. These data imply that H_2S can play a role as downstream signal in the vasodilatory effect of testosterone, but they did not represent a direct proof that testosterone can cause H_2S release and/or generation. In order to address this issue, we evaluated if incubation of aorta tissue with testosterone could induce H_2S production and/or increase the conversion of the substrate L-cysteine to H_2S. In the aorta homogenate following incubation with testosterone, there was no detectable increase in the basal release of H_2S. Conversely, when homogenate was challenged with L-cysteine, there was a significant increase of H_2S production after 30 and 60 min of incubation. The finding that H_2S levels after 5 and 15 min were not different seems to be an apparent discrepancy with functional data where the concentration-response curve of testosterone lasts 10–30 min. This

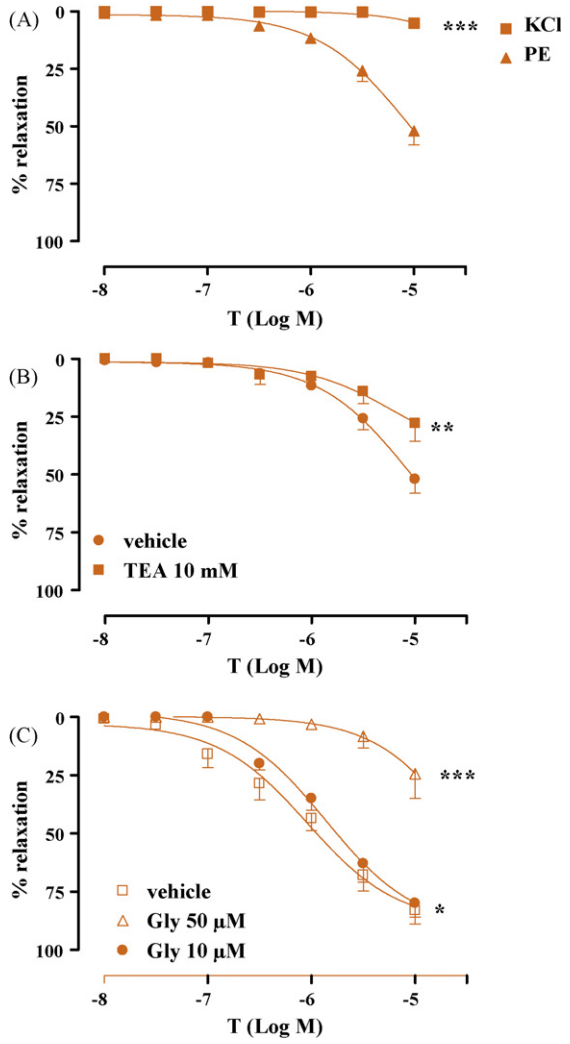

Fig. 5 – Panel A: Effect of testosterone on KCl (60 mM) pre-contracted rings; panel B: effect of TEA (10 mM); and panel C: effect of glybenclamide (10, 50 μM) on testosterone-induced vasorelaxation. *** $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, $n = 6$ for each group.

apparent discrepancy reflects the limit of the assay, whose lower limit is 3 μM. In other words, most likely the concentration of H_2S necessary to obtain the effect in vitro in the functional experiments is out of the detection limit of the assay. The finding that T incubation did not enhance the basal release of H_2S but significantly enhanced L-cysteine-stimulated production of H_2S suggested a possible positive modulation of CSE/CBS activity by testosterone rather than an increase in enzyme expressions. To explore this issue, we performed the in vitro assay in the presence of either PGG or BCA. Both BCA and PGG significantly inhibited T-induced H_2S production. Interestingly, both inhibitors reversed H_2S levels to the control value, further suggesting that testosterone effect is on the enzyme activity rather than on the enzyme expressions. This last hypothesis was confirmed by the Western blot analysis performed on tissues exposed to testosterone where the expression of either CBS or CSE was not modified. Thus, in our experimental conditions and in the early time frame considered in our study, testosterone increases CSE/CBS activity rather than their relative expression.

Having determined a role for H_2S in testosterone-induced vasodilatation, we addressed the other point we aimed to confirm: the involvement of K_{ATP} channels in our experimental conditions. Indeed, H_2S activity has been linked to potassium channels activation and in particular to K_{ATP} channels [20,26–28]. We first addressed the possible involvement of potassium conductance, comparing testosterone cumulative concentration-response curve following contraction with either KCl or PE. When rings were contracted using the depolarizing effect of KCl, testosterone-induced vasodilatation was abrogated, indicating the involvement of potassium conductance. This finding is in line with the literature, where it has been clearly shown that calcium-activated and/or large conductance calcium-activated K^+ channels are involved in testosterone-induced vasorelaxation [10,12,29]. This finding was further supported by the finding that TEA significantly inhibited testosterone-induced vasorelaxation. However, since TEA is known to block different types of K^+ channels [22] we used glybenclamide, a specific inhibitor of K^+_{ATP} channels. Glybenclamide inhibited testosterone vasodilator action in a concentration-dependent manner, confirming that K_{ATP} channels are the major player in our experimental conditions. Thus, testosterone-induced vasorelaxation involves H_2S biosynthesis that in turn activates K_{ATP} channels.

5. Conclusions

Several studies have shown that acute administration of testosterone induces a rapid relaxation in vascular tissues of different species, including humans, suggesting a nongenomic effect of this hormone on vascular reactivity. Different mechanisms have been proposed to explain testosterone-induced vasodilatation, but is the effective mechanism(s) and the mediators are a still matter of debate. Here we have shown that the testosterone nongenomic vascular effect involves the L-cysteine/H_2S pathway. In particular, the data presented demonstrate that, in rat aorta, the nongenomic effect of testosterone is linked to a positive modulation of CSE/CBS activity. The H_2S produced acts on K_{ATP} channels, contributing to the vasodilator effect of testosterone. Thus, H_2S involvement in the vascular activity of testosterone may help to explain the beneficial effects of testosterone on the cardiovascular system.

Author contributions: Giuseppe Cirino had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Cirino, Mirone, Imbimbo.
Acquisition of data: Bucci, Di Lorenzo, Vellecco, Roviezzo.
Analysis and interpretation of data: Bucci, Roviezzo, Cirino.
Drafting of the manuscript: Cirino, Bucci.
Critical revision of the manuscript for important intellectual content: Mirone, Imbimbo, Cirino.

Statistical analysis: Bucci, Brancaleone.
Obtaining funding: None.
Administrative, technical, or material support: Velleco, Di Lorenzo.
Supervision: Cirino.
Other (specify): None.

Financial disclosures: I certify that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/ affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

Funding/Support and role of the sponsor: None.

References

[1] English KM, Mandour O, Steeds RP, Diver MJ, Jones TH, Channer KS. Men with coronary artery disease have lower levels of androgens than men with normal coronary angiograms. Eur Heart J 2000;21:890–4.

[2] Hromadova M, Hacik T, Malatinsky E, Riecansky I. Alteration of lipid metabolism in men with hypotestosteronemia. Horm Metabol Res 1991;23:392–4.

[3] Eckardstein A, Wu FCW. Testosterone and atherosclerosis. Growth Horm & IGF Res 2003;13:S72–84.

[4] English KM, Steeds R, Jones TH, Channer KS. Testosterone and coronary heart disease: is there a link? Q J Med 1997;90:787–91.

[5] Smith AM, Jones RD, Channer KS. The influence of sex hormones on pulmonary vascular reactivity: possible vasodilator therapies for the treatment of pulmonary hypertension. Curr Vasc Pharmacol 2006;4:9–15.

[6] Jones RD, Nettleship JE, Kapoor D, Jones HT, Channer KS. Testosterone and atherosclerosis in aging men: purported association and clinical implications. Am J Cardiovasc Drugs 2005;5:141–54.

[7] Chou TM, Sudhir K, Hutchison SJ, et al. Testosterone induces dilatation of canine coronary conductance and resistance arteries in vivo. Circulation 1996;94:2614–9.

[8] Costarella CE, Stallone JN, Rutecki GW, Whittier FC. Testosterone causes direct relaxation of rat thoracic aorta. J Pharm Exp Ther 1996;277:34–9.

[9] Perusquia M, Hernandez R, Morales MA, Campos MG, Villalon MG. Role of the endothelium in the vasodilating effect of progestins and androgens on the rat thoracic aorta. Gen Pharmacol 1996;27:181–5.

[10] Honda H, Unemoto T, Kogo H. Different mechanisms for testosterone-induced relaxation of aorta between normotensive and spontaneously hypertensive rats. Hypertension 1999;34:1232–6.

[11] Crews JK, Khalil RA. Antagonistic effect of 17 beta-oestradiol, progesterone and testosterone on Ca^{2+} entry mechanism of coronary vasoconstriction. Arterioscler Thromb Vasc Biol 1999;19:1034–40.

[12] Tep-areenan P, Kendall DA, Randall MD. Testosterone-induced vasorelaxation in the rat mesenteric arterial bed is mediated predominantly via potassium channels. Br J Pharmacol 2002;135:735–40.

[13] Levonen AL, Lapatto R, Saksela M, Raivio KO. Human cystathionine gamma-lyase: developmental and in vitro expression of two isoforms. Biochem J 2000;347:291–5.

[14] Yap S, Naughten ER, Wilcken B, Wilcken DE, Boers GH. Vascular complications of severe hyperhomocysteinemia in patients with homocystinuria due to cystathionine beta synthase deficiency: effects of homocysteine-lowering therapy. Semin Thromb Hemost 2000;26:335–40.

[15] Van der Molen EF, Hiipakka MJ, van Lith-Zanders H, et al. Homocysteine metabolism in endothelial cells of a patient homozygous for cystathionine beta-synthase (CS) deficiency. Thromb Haemost 1997;78:827–33.

[16] Zhao W, Ndisang JF, Wang R. Modulation of endogenous production of H_2S in rat tissues. Can J Physiol Pharmacol 2003;81:848–53.

[17] Hosoki R, Matsiki N, Kimura H. The possible role of hydrogen sulphide as an endogenous smooth muscle relaxant in synergy with nitric oxide. Biochem Biophysic Res Comun 1997;237:527–31.

[18] Yue P, Chatterjee K, Beale C, Poole-Wilson PA, Collins P. Testosterone relaxes rabbit coronary arteries and aorta. Circulation 1995;91:1154–60.

[19] Ding AQ, Stallone JN. Testosterone-induced relaxation of rat aorta is androgen structure specific and involves K^+ channel activation. J Appl Physiol 2001;91:2742–50.

[20] Zhao W, Zhang J, Lu Y, Wang R. The vasorelaxant effect of H_2S as a novel endogenous gaseous K_{ATP} channel opener. EMBO J 2001;20:6008–16.

[21] Cheng Y, Ndisang JF, Tang G, Cao K, Wang R. Hydrogen sulphide-induced relaxation of resistance mesenteric artery beds of rats. Am J Physiol Heart Circ Physiol 2004;287:H2316–23.

[22] Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol 1995;268:C799–822.

[23] Reed DJ. Cystathionine. Meth In Enzym 1995;252:92–102.

[24] Uren JR, Ragin R, Chaykovsky M. Modulation of cysteine metabolism in mice—effects of propargylglycine and L-cysteine-degrading enzymes. Bioche Pharmacol 1978;27:2807–14.

[25] Stipanuk MH, Beck PW. Characterization of the enzymatic capacity for cysteine desulphydration in liver and kidney of the rat. Biochem J 1982;206:267–77.

[26] Szabo C. Hydrogen sulphide and its therapeutic potential. Available at http://www.nature.com/nrd/journal/v6/n11/abs/nrd2425.html.

[27] Johansen D, Ytrehus K, Baxter GF. Exogenus hydrogen sulphide (H_2S) protects against regional myocardial ischemia-reperfusion injury. Evidence for a role of K ATP channels. Basic Res Cardiol 2006;101:53–60.

[28] Fiorucci S, Antonelli E, Mencarelli A, et al. The third gas: H_2S regulates perfusion pressure in both the isolated and perfused normal rat liver and in cirrhosis. Hepatology 2005;42:539–48.

[29] Jones RD, English KM, Jones TH, Channer KS. Testosterone-induced coronary vasodilatation occurs via non-genomic mechanism: evidence of a direct calcium antagonism action. Clin Sci (Lond) 2004;107:149–58.

Editorial Comment on: Hydrogen Sulphide Is Involved in Testosterone Vascular Effect

Andrea Salonia
Department of Urology, Scientific Institute H. San Raffaele, Milan, Italy
saloniaandrea@yahoo.com

The "love story" between testosterone and sexual medicine began several years ago. Suddenly, the "revolutionary blue pill" arrived, and we had the probably erroneous presumption to potentially treat or cure each patient using the same oral compound. It was not to be: phosphodiesterase type 5 inhibitors

(PDE5-Is) by themselves are not always a "magic" cure [1].

In this scenario, based on the preclinical and clinical data available in the literature to date, it is possible to infer that androgens are essential for the development and growth of the penis, and they play a critical role in maintaining erectile physiology in men [2,3]. On the basis of the evidence derived from laboratory animal studies and the most up-to-date clinical data, it has been postulated that an androgen insufficiency may disrupt cellular signalling pathways and produce pathologic alterations in penile tissue, finally leading to erectile dysfunction (ED) [3]. Vascular physiology and penile haemodynamics—namely the functional properties of the penile tissue throughout the erection process—certainly deserve an adequate androgen milieu. In this context, circulating testosterone has been proven to have a nongenomic effect towards vascular reactivity, promoting a rapid relaxation of the vascular beds.

How does testosterone have this effect? Different mechanisms have been proposed [4,5]. The present study by Bucci et al [6] elegantly suggests that testosterone might increase the overall production of hydrogen sulphide, a gaseous modulator with affinity for, at least, the rat vascular system, both in vivo and in vitro. But even the famous nitric oxide (NO) is a gas, isn't it? Do you remember the functional pathway for sildenafil and similar drugs? It certainly deserves the activation of the NO–cyclic guanosine monophosphate (cGMP) pathway to properly work, thus promoting a penile erection [7]. Therefore, from a clinical standpoint, it is important to once more highlight that screening for hypogonadism in men with ED is certainly necessary to identify those with an inadequate circulating testosterone level who may benefit from testosterone substitution [1,8]. Identification of threshold values for testosterone supplementation has been rigorously reported [8], and convincing data has shown who should actually be appropriately treated with a combined therapy when PDE5-Is alone fail.

Fortunately, research in sexual medicine can still move forward!

References

[1] Greco EA, Spera G, Aversa A. Combining testosterone and PDE5 inhibitors in erectile dysfunction: basic rationale and clinical evidences. Eur Urol 2006;50:940–7. Corrigendum. Eur Urol 2007;51:872.

[2] Traish AM, Guay AT. Are androgens critical for penile erections in humans? Examining the clinical and preclinical evidence. J Sex Med 2006;3:382–404.

[3] Traish AM, Goldstein I, Kim NN. Testosterone and erectile function: from basic research to a new clinical paradigm for managing men with androgen insufficiency and erectile dysfunction. Eur Urol 2007;52:54–70.

[4] Han DH, Chae MR, Jung JH, So I, Park JK, Lee SW. Effect of testosterone on potassium channel opening in human corporal smooth muscle cells. J Sex Med 2008;5:822–32.

[5] Vignozzi L, Morelli A, Filippi S, et al. Testosterone regulates RhoA/Rho-kinase signaling in two distinct animal models of chemical diabetes. J Sex Med 2007;4:620–30.

[6] Bucci M, Mirone V, Di Lorenzo A, et al. Hydrogen sulphide is involved in testosterone vascular effect. Eur Urol 2009;56:378–84.

[7] Goldstein I, Lue TF, Padma-Nathan H, Rosen RC, Steers WD, Wicker PA. Oral sildenafil in the treatment of erectile dysfunction. Sildenafil Study Group. N Engl J Med 1998;338:1397–404.

[8] Isidori AM, Giannetta E, Gianfrilli D, et al. Effects of testosterone on sexual function in men: results of a meta-analysis. Clin Endocrinol (Oxf) 2005;63:381–94.

DOI: 10.1016/j.eururo.2008.05.015

DOI of original article: 10.1016/j.eururo.2008.05.014