Nebivolol Potentiates the Efficacy of PDE5 Inhibitors to Relax Corpus Cavernosum and Penile Arteries from Diabetic Patients by Enhancing the NO/cGMP Pathway

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ABSTRACT

Introduction. The efficacy of oral pharmacotherapy for erectile dysfunction (ED) (i.e., type 5 phosphodiesterase [PDE5] inhibitors) is significantly reduced in diabetic patients. Nebivolol is a selective β_1-blocker used for treating hypertension that has been shown to increase the efficacy of sildenafil to reverse ED in diabetic rats.

Aim. To evaluate the effects of nebivolol on the efficacy of the PDE5 inhibitors, sildenafil, tadalafil, and vardenafil to relax human corpus cavernosum (HCC) and vasodilate human penile resistance arteries (HPRA) from diabetic patients with ED (DMED). The influence of nebivolol on the capacity of these three PDE5 inhibitors to stimulate cyclic guanosine monophosphate (cGMP) production in HCC was also evaluated.

Methods. HCC and HPRA were obtained from organ donors without ED (NEND; n = 18) or patients with diabetes undergoing penile prosthesis implantation (DMED; n = 19). Relaxations of HCC strips and HPRA to sildenafil, tadalafil, and vardenafil were evaluated in organ chambers and wire myographs. cGMP content in HCC was determined by ether extraction and quantification by ELISA.

Main Outcome Measures. Effects of nebivolol on PDE5 inhibitor-induced relaxation of HCC, vasodilation of HPRA and cGMP accumulation in HCC.

Results. Treatment with nebivolol (1 μM) significantly potentiated sildenafil-, tadalafil- and vardenafil-induced relaxations of HCC and vasodilations of HPRA from both NEND and DMED. Enhancement of relaxant capacity by nebivolol resulted in reversion of the impairment of PDE5 inhibition-induced responses in DMED and it was accompanied by enhancing the ability of PDE5 inhibitors to increase cGMP in HCC restoring reduced cGMP levels in HCC from DMED.

Conclusions. Nebivolol potentiated the capacity of PDE5 inhibitors to relax vascular structures of erectile tissue from diabetic patients by enhancing the nitric oxide (NO)/cGMP pathway in these tissues. These effects suggest a potential therapeutic utility of nebivolol as an adjunct to PDE5 inhibitors for the treatment of ED associated with diabetes. **Martínez-Salamanca JI, La Fuente JM, Cardoso J, Fernández A, Cuevas P, Wright HM, and Angulo J. Nebivolol potentiates the efficacy of PDE5 inhibitors to relax corpus cavernosum and penile arteries from diabetic patients by enhancing the NO/cGMP pathway. J Sex Med 2014;11:1182–1192.**

Key Words. Nebivolol; Erectile Dysfunction and Hypertensive Agents; Nitric Oxide; β-Blockers; Type 5 Phosphodiesterase Inhibitors; Diabetes; Corpus Cavernosum and Penile Arteries

Introduction

Relaxation of trabecular smooth muscle and dilation of human penile resistance arteries (HPRA) within the corpora cavernosa upon sexual stimulation are two necessary events for penile erection [1]. Nitric oxide (NO) is a key factor in both events. Either released from nerve terminals or endothelial cells, NO stimulates cyclic guanosine monophosphate (cGMP) production in penile smooth muscle cells causing its relaxation and increasing blood flow into the corpora cavernosa [2,3]. Any defect in NO/cGMP pathway at any level would result in inadequate penile smooth muscle relaxation and compromise erectile function [4].

Erectile dysfunction (ED) is highly prevalent among diabetic men [5]. The presence of ED is associated with lower scores of Diabetes Quality-of-Life questionnaire in long-term follow-up of type 1 diabetic patients [6]. In fact, ED is associated with an increased risk for cardiovascular events in diabetic patients [7] and the prognostic utility of ED for cardiovascular diseases (CVD) is particularly high in diabetic patients [8]. Importantly, diabetic patients are particularly resistant to the conventional treatment of ED (i.e., type 5 phosphodiesterase (PDE5) inhibitors) [9,10]. Thus, the search for alternative pharmacologic strategies for improving the efficacy of available treatments for diabetic ED is definitely justified.

Nebivolol is a highly selective β_1-adrenoceptor antagonist with NO–mediated vasodilatory properties [11,12] that, unlike traditional β-blockers, such as atenolol and metoprolol, is associated with few or no reported ED-related adverse events [13,14]. Hypertensive men treated with nebivolol do not demonstrate decreases in their International Index of Erectile Function (IIEF) scores; in fact, they show improvements in secondary sexual activity scores and other IIEF subscores, compared to men treated with metoprolol [15]. Similar positive findings have been demonstrated with nebivolol when compared to atenolol and other β-blockers [16,17]. Furthermore, the American Heart Association recommend nebivolol when patients present sexual dysfunction associated with the administration of β-blockers provided the β-blocker is not being administered specifically for survival improvement for the patient with systolic heart failure or after myocardial infarction [18]. The potential benefit of nebivolol on erectile function in humans is likely related to its ability, not shared by other β_1-adrenoceptor antagonists, to increase NO bioavailability, increasing expression/activity of endothelial NO synthase (eNOS) and improving endothelial function in corpus cavernosum from rodents [19–21]. Human corpora cavernosa (HCC) and penile arteries from diabetic patients display an exacerbated deficit of NO/cGMP pathway that could be responsible for the reduced efficacy of PDE5 inhibitors [22]. Thus, an extra supply of NO by nebivolol would potentially improve the efficacy of PDE5 inhibitors to relax diabetic erectile tissue and facilitate erection. In this sense, sustained administration of nebivolol was able to improve erectile responses in diabetic rats, reaching a complete reversion of ED when combined with the PDE5 inhibitor, sildenafil, through increased cGMP levels in penile tissue from these animals [23].

Based on these data, the aim of this work was to evaluate the effects of nebivolol on the efficacy of the PDE5 inhibitors, sildenafil, tadalafil and vardenafil to relax HCC and vasodilate HPRA from diabetic patients with ED. We also evaluated the influence of nebivolol on the capacity of these three PDE5 inhibitors to stimulate cGMP production in HCC.

Methods

Human Tissues

Human penile tissue biopsies were obtained from 18 organ donors with no reported history of diabetes or ED and from 19 type 2 diabetic patients with ED who gave informed consent at the time of penile prosthesis insertion. In addition to their diabetes, hypertension was present in 10 patients and hypercholesterolemia in two patients. Aetiology of ED was considered as vascular in 11 patients, neurological in 6 patients (4 patients after radical prostatectomy) and mixed in 2 patients. ED secondary to Peyronie's disease was diagnosed in one patient. Diabetic patients with ED were significantly older that organ donors (62.3 ± 1.4 vs. 52.8 ± 4.1 years, $P < 0.05$). Organ donors were free of obesity and dyslipidemias and only 2 out of 18 presented elevated blood pressure while not taking medication. Tissues were collected at Hospital Universitario Puerta de Hierro, Madrid, Spain and Hospital Santo Antonio, Porto, Portugal. Ethics Committees from both institutions approved the study. Tissues were maintained at 4°C to 6°C in M-400 solution (composition per 100 mL: mannitol, 4.19 g; KH_2PO_4, 0.205 g; $K_2HPO_4{\cdot}3H_2O$, 0.97 g; KCl, 0.112 g; $NaHCO_3$, 0.084 g) until used, which was between 2 and 16 hours after extraction [22].

Corpus Cavernosum

Strips of corpus cavernosum tissue (3 × 3 × 7 mm) were immersed in 8-mL organ chambers containing physiologic salt solution (PSS) of the following composition: NaCl, 119 mM; KCl, 4.6 mM; $CaCl_2$, 1.5 mM; $MgCl_2$, 1.2 mM; $NaHCO_3$, 24.9 mM; glucose, 11 mM; KH_2PO_4, 1.2 mM; and EDTA, 0.027 mM. The strips were maintained at 37°C and aerated with 5% CO_2/95% O_2, pH 7.4. Each tissue strip was incrementally stretched to optimal isometric tension, as determined by maximal contractile response to 1 μM of phenylephrine. The preparations were then exposed to 120 mM K^+ (KPSS: equimolar substitution of NaCl for KCl in PSS), and the contractile response was measured. HCC strips displaying KPSS-induced contractions below 0.2 g were discarded. Strips were contracted with the thromboxane receptor agonist U46619 (1 nM to 3 nM; 80% of KPSS-induced contraction, approximately), and relaxation response was evaluated by cumulative additions of compounds to the chambers. U46619 was chosen to avoid potential interferences of nebivolol with adrenergic agonists. Nebivolol (1 μM) or vehicle were added 20 minutes before contraction with U46619 for evaluating sildenafil-, tadalafil- and vardenafil-induced relaxations.

Penile Resistance Arteries

Penile small helicine arteries (lumen diameter 150 μm to 400 μm), which are the terminal branches of deep penile arteries, were dissected by carefully removing the adhering trabecular tissue, and arterial ring segments (2-mm long) were subsequently mounted on microvascular wire myographs (J.P. Trading; Aarhus, Denmark) for isometric tension recordings [22,24]. The vessels were allowed to equilibrate for 30 minutes in PSS at 37°C, continuously bubbled with 95% O_2/5% CO_2 mixture to maintain a pH of 7.4. Passive tension and internal circumference of vascular segments when relaxed *in situ* under a transmural pressure of 100 mm Hg (L_{100}) were determined. The arteries were then set to an internal circumference equivalent to 90% of L_{100}, at which the force development was close to maximal [25]. The preparations were then exposed to 120 mM K^+ (KPSS) and the contractile response was measured. HPRA segments failing to produce a tension equivalent to a pressure of 100 mm Hg were rejected. The arteries were contracted with 10 nM to 30 nM of U46619 (80% of KPSS-induced contraction, approximately), and relaxation response was evaluated by cumulative additions of compounds to the chambers. Nebivolol (1 μM) or vehicle were added 20 minutes before contraction with U46619 for evaluating sildenafil-, tadalafil-, and vardenafil-induced responses. Sildenafil (30 nM), tadalafil (30 nM) and vardenafil (10 nM) were added 30 minutes before contraction with U46619 for evaluating nebivolol-induced relaxations. The presence of functional endothelium was previously confirmed in all arterial preparations by assessing relaxation to acetylcholine (ACh; 10 μM).

Determination of cGMP Content in Human Cavernosal Tissues

After administering the respective treatments, HCC strips were immediately frozen by immersion in liquid nitrogen and stored at –80°C until extraction for cyclic nucleotides. Tissues were extracted by homogenization in 6% trichloroacetic acid, followed by ether (H_2O-saturated) extraction and lyophilization. The concentration of cGMP was determined by enzyme-linked immunosorbent assay, using a kit from Cayman Chemical Company (Ann Arbor, MI, USA) [22].

Drugs and Materials

Phenylephrine, and acetylcholine chloride were obtained from Sigma-Aldrich (St. Louis, MO, USA). 9,11-dideoxy-9α,11α-epoxymethano $PGF_{2\alpha}$ (U46619) was obtained from Alexis Corporation (Lausen, Switzerland). Sildenafil was a gift from Nitromed (Bedford, MA, USA) while tadalafil was provided by ICOS Corporation (Seattle, WA, USA) and vardenafil was provided by Bayer AG (Wuppertal, Germany). d,l-nebivolol HCl was provided by Forest Laboratories, Inc. (New York, NY, USA). Nebivolol, sildenafil, tadalafil and vardenafil were dissolved at 10 mM concentration in dimethylsulfoxide (DMSO). The subsequent dilutions were made in deionized water. Final DMSO concentrations were 1% or lower. All other drugs were dissolved in deionized water.

Data Analyses

Data are expressed as mean ± standard error. Relaxation response is expressed as the percentage of total relaxation (i.e., loss of contractile tone achieved with exposure to U46619). Complete concentration-response curves were obtained and compared by a two-factor analysis of variance

(ANOVA) (StatView, SAS; Cary, NC, USA). Individual concentrations and cGMP data were compared by one-factor ANOVA, followed by a Student-Newmann-Keuls post-test (GraphPad InStat; San Diego, CA, USA). E_{max} indicates maximum relaxation response. pD_2 is defined as –log M of the concentration required to obtain 50% of maximum relaxation.

Results

Effect of Nebivolol on Relaxations Induced by Sildenafil, Tadalafil, and Vardenafil in HCC from Diabetic Patients

Contractile capacity of HCC was not altered by diabetes since KPSS induced contractions were not significantly different between HCC strips from NEND and DMED (5.55 ± 0.53 g vs. 4.85 ± 0.64 g, respectively). Cumulative additions of PDE5 inhibitors resulted in concentration-dependent relaxation of U46619-precontracted HCC strips from both organ donors without a history of ED and diabetes (NEND) and diabetic patients with ED (DMED). Relaxations induced by sildenafil, tadalafil and vardenafil (1 nM to 10 μM) were significantly reduced in HCC from DMED vs. NEND patients (Figure 1). The three PDE5 inhibitors displayed similar potency for relaxing HCC, although vardenafil was slightly and non-significantly superior (pD_2 7.42 ± 0.17, 7.36 ± 0.12, and 7.73 ± 0.15 for sildenafil, tadalafil and vardenafil, respectively, in NEND, and pD_2 6.82 ± 0.29, 6.84 ± 0.21, and 7.08 ± 0.43 for sildenafil, tadalafil and vardenafil, respectively, in DMED). Treatment of HCC with nebivolol (1 μM) resulted in significant potentiation of the relaxation induced by the PDE5 inhibitors, sildenafil, tadalafil or vardenafil (1 nM to 10 μM)

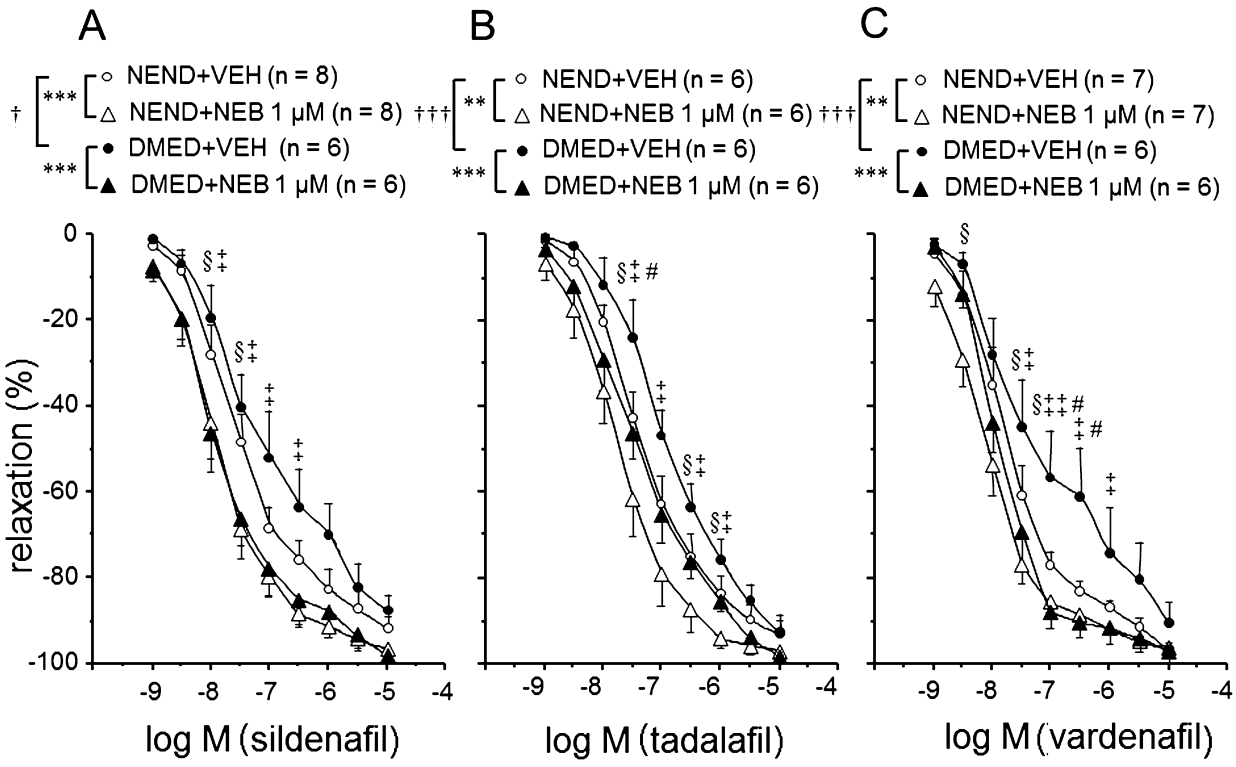

Figure 1 Nevibolol potentiates the relaxant capacity of PDE5 inhibitors in human corpus cavernosum (HCC) from diabetic patients with ED.

Effects of nebivolol (NEB; 1 μM) or vehicle (VEH; 0.01% DMSO) on relaxations induced by the PDE5 inhibitors (1 nM to 10 μM), sildenafil (A), tadalafil (B), and vardenafil (C), in human corpora cavernosa strips from organ donors without a history of diabetes or ED (NEND) and from diabetic patients with ED (DMED) contracted with the thromboxane analogue U46619 (1 nM to 3 nM). Data are expressed as mean ± SEM of the percentage of relaxation. n indicates the number of patients from whom the tissues were collected. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. VEH, $\dagger P < 0.05$, $\dagger\dagger\dagger P < 0.001$ vs. NEND by two-factor ANOVA. $\S P < 0.05$ NEB vs. VEH in NEND, $\ddagger P < 0.05$ NEB, $\ddagger\ddagger P < 0.01$ vs. VEH in DMED, $\# P < 0.05$ NEND+VEH vs. DMED+VEH by one-factor ANOVA followed by Student-Newmann-Keuls test.

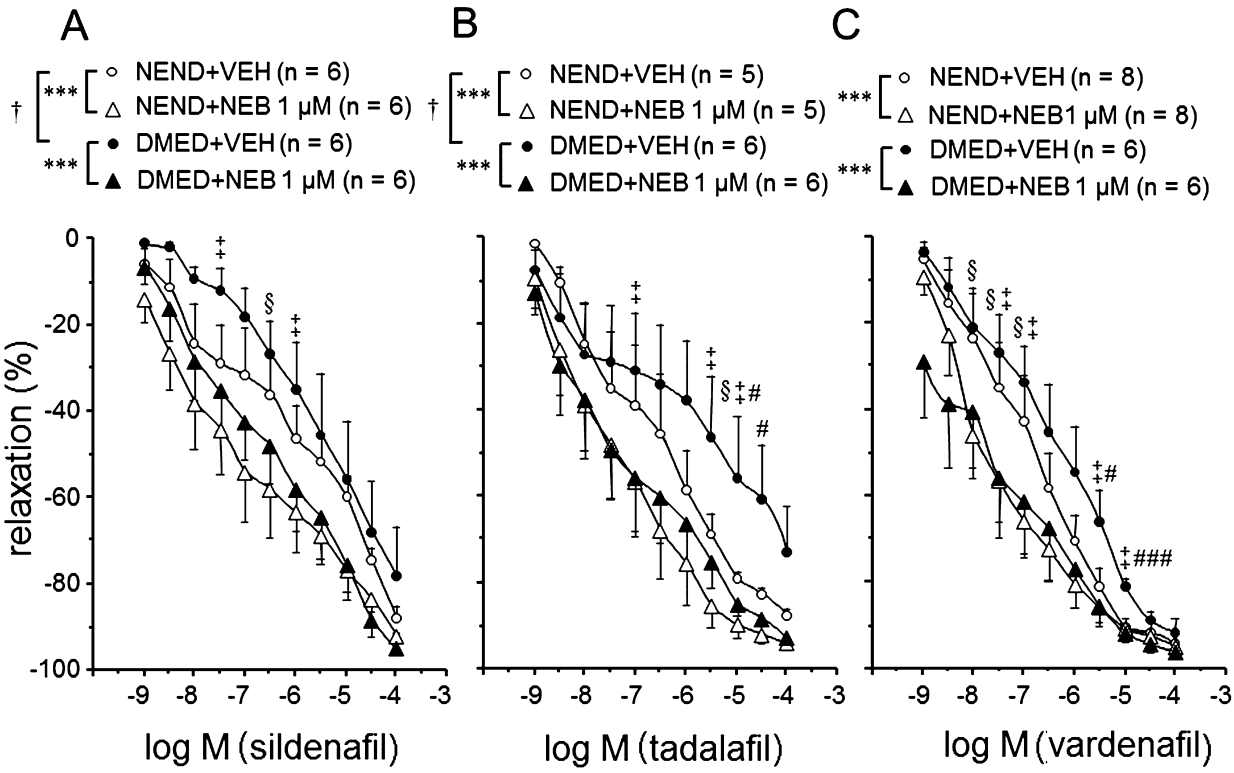

Figure 2 Nevibolol potentiates the vasodilatory capacity of PDE5 inhibitors in human penile resistance arteries (HPRA) from diabetic patients with ED.
Effects of nebivolol (NEB; 1 μM) or vehicle (VEH; 0.01% DMSO) on relaxations induced by the PDE5 inhibitors (1 nM to 100 μM), sildenafil (A), tadalafil (B), and vardenafil (C), in HPRA from organ donors without a history of diabetes or ED (NEND) and from diabetic patients with ED (DMED) contracted with the thromboxane analogue U46619 (10 nM to 30 nM). Data are expressed as mean ± SEM of the percentage of relaxation. n indicates the number of patients from whom the tissues were collected. ***$P < 0.001$ vs. VEH, †$P < 0.05$ vs. NEND by two-factor ANOVA. §$P < 0.05$ NEB vs. VEH in NEND, ‡$P < 0.05$ NEB vs. VEH in DMED, #$P < 0.05$, ###$P < 0.001$ NEND+VEH vs. DMED+VEH by one-factor ANOVA followed by Student-Newmann-Keuls test.

in HCC strips from both NEND and DMED patients (Figure 1). After nebivolol treatment, PDE5 inhibitor-induced relaxations in HCC from DMED were not inferior to that of vehicle treated HCC from NEND subjects. In fact, a significantly greater relaxation was observed for sildenafil (Figure 1C).

Effect of Nebivolol on Relaxations Induced by Sildenafil, Tadalafil, and Vardenafil in Human Penile Arteries from Diabetic Patients

Similarly to that observed in HCC, KPSS-induced contractions in HPRA segments from NEND and DMED were not significantly different (7.87 ± 0.77 mN vs. 8.84 ± 1.01 mN, respectively). In U46619-precontracted HPRA, increasing concentrations of the PDE5 inhibitors evoked concentration-dependent vasodilations of HPRA from both NEND and DMED patients. These vasodilations induced by sildenafil, tadalafil and vardenafil (1 nM to 100 μM) were reduced in HPRA from DMED, although statistically significant only for sildenafil and tadalafil (Figure 2). No significant differences in potency among the three PDE5 inhibitors, were observed in NEND or DMED patients, although sildenafil tended to display lower potency (pD_2 5.96 ± 0.59, 7.09 ± 0.12, 6.89 ± 0.15 for sildenafil, tadalafil and vardenafil, respectively, in NEND, and pD_2 5.22 ± 0.44, 5.70 ± 0.58, 6.47 ± 0.45 for sildenafil, tadalafil and vardenafil, respectively, in DMED). Nebivolol (1 μM) exerted significant potentiation of the vasodilation induced by the PDE5 inhibitors, sildenafil, tadalafil or vardenafil (1 nM to 100 μM) in HPRA from both NEND and DMED patients (Figure 2). All existing impairment of PDE5 inhibitor-induced vasodilations in HPRA

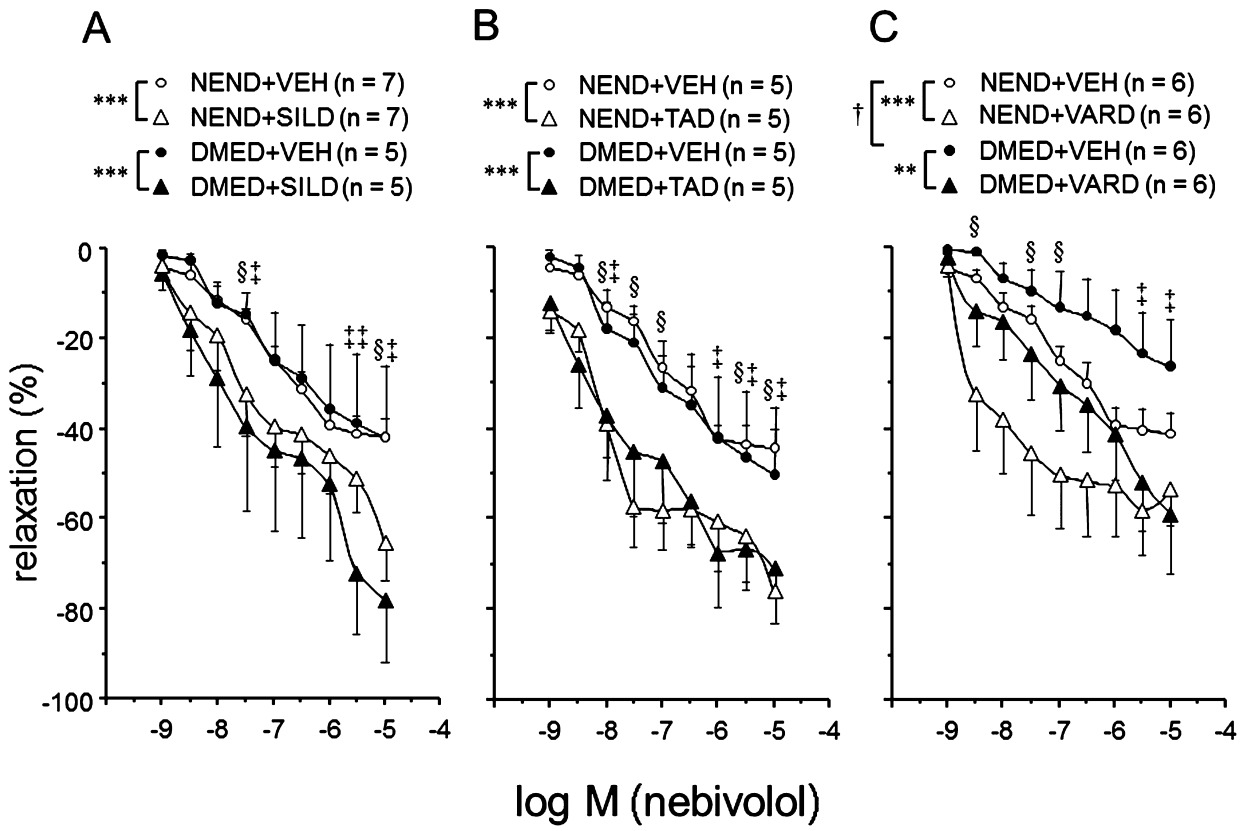

Figure 3 PDE5 inhibitors enhance vasodilation induced by nebivolol in human penile resistance arteries (HPRA) from diabetic patients with ED.
Effects of sildenafil (SILD; 30 nM) (A), tadalafil (TAD; 30 nM) (B), vardenafil (VARD; 10 nM) (C) or vehicle (VEH; 0.01% DMSO) on relaxations induced by nebivolol (1 nM to 10 μM) in HPRA from organ donors without a history of diabetes or ED (NEND) and from diabetic patients with ED (DMED) contracted with the thromboxane analogue U46619 (10 nM to 30 nM). Data are expressed as mean ± SEM of the percentage of relaxation. n indicates the number of patients from whom the tissues were collected. ***$P < 0.001$ vs. VEH, †$P < 0.05$ vs. NEND by two-factor ANOVA. §$P < 0.05$ PDE5 inhibitor vs. VEH in NEND, ‡$P < 0.05$, ‡‡$P < 0.01$ PDE5 inhibitor vs. VEH in DMED by one-factor ANOVA followed by Student-Newmann-Keuls test.

from DMED with respect to NEND were corrected after nebivolol treatment (Figure 2).

Effect of Sildenafil, Tadalafil, and Vardenafil on Vasodilatory Capacity of Nebivolol in HPRA

Nebivolol (1 nM to 100 μM) did not cause significant relaxation of HCC (E_{max} 13.4 ± 2.7% vs. 15.1 ± 8.1% for vehicle and nebivolol, respectively) but nebivolol (1 nM to 10 μM) exerted concentration-dependent vasodilations of U46619-contracted HPRA from both NEND and DMED patients (Figure 3). Although in HPRA segments used for vardenafil experiments nebivolol-induced relaxations were significantly reduced in DMED, analysis of all vasodilatory responses exerted by nevibolol yielded no significant differences between NEND and DMED (E_{max} 45.0 ± 4.2% vs. 40.6 ± 12.0% in NEND and DMED, respectively). These vasodilations were significantly potentiated by inhibiting PDE5 with either sildenafil (30 nM), tadalafil (30 nM) or vardenafil (10 nM). This potentiation was produced in HPRA from both NEND and DMED patients (Figure 3).

Effect of Nebivolol on PDE5 Inhibitor-Induced cGMP Accumulation in HCC from Diabetic Patients

After exposure to sildenafil, tadalafil, and vardenafil (10 μM), cGMP accumulation was reduced in HCC from DMED patients when compared to NEND, a reduction that was statistically significant for sildenafil and tadalafil (Figure 4). Treatment with nebivolol (1 μM) resulted in enhanced cGMP accumulation in

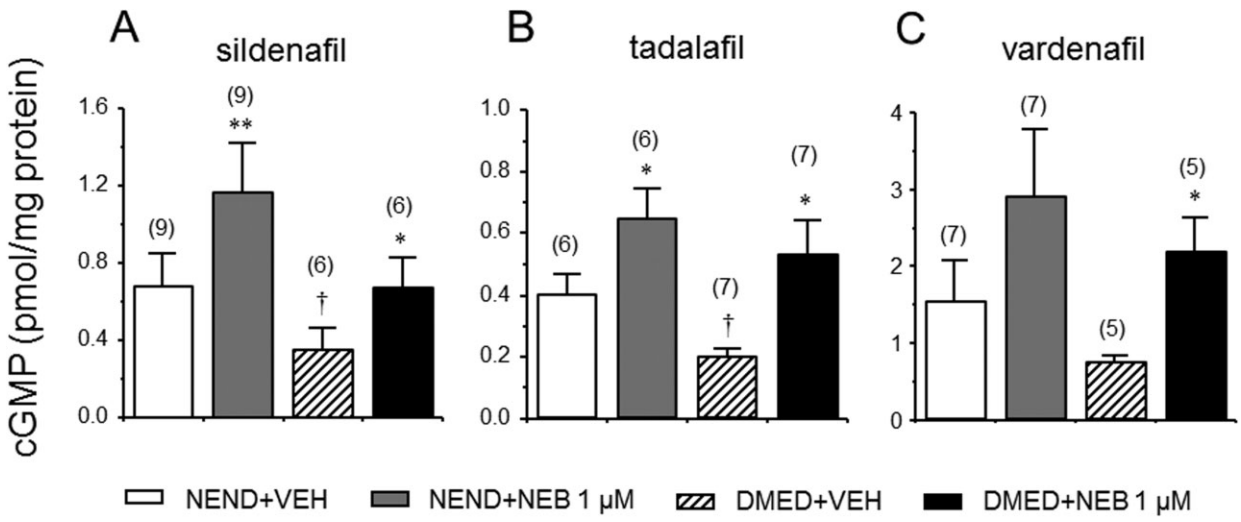

Figure 4 Nebivolol increases cGMP accumulation induced by PDE5 inhibitors in human corpus cavernosum from diabetic patients with ED.
Effects of nebivolol (NEB; 1 μM) or vehicle (VEH; 0.01% DMSO) on cGMP accumulation induced by the PDE5 inhibitors (10 μM), sildenafil (A), tadalafil (B), and vardenafil (C), in human corpus cavernousm from organ donors without a history of diabetes or ED (NEND) and from diabetic patients with ED (DMED). Data are expressed as mean±SEM of pmoles of cGMP per milligram of tissue protein. n indicates the number of patients from whom the tissues were collected. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle, $\dagger P < 0.05$ vs. NEND by one-factor ANOVA followed by Student-Newmann-Keuls test.

HCC from both NEND and DMED patients in response to PDE5 inhibitor exposure (Figure 4). With the exception of vardenafil in HCC from NEND, this increase in cGMP reached statistical significance (Figure 4C). In all cases, cGMP levels induced by PDE5 inhibitors in HCC from DMED in the presence of nebivolol were not different from that achieved after PDE5 inhibition in vehicle-treated NEND tissues (Figure 4).

Discussion

The present results demonstrate that nebivolol enhances the capacity of PDE5 inhibitors to relax HCC and to vasodilate human penile arteries. This potentiation is observed in healthy tissues as well as in erectile tissues from diabetic patients with ED, a population less responsive to PDE5 inhibition therapy. Furthermore, the enhancement driven by nebivolol is applicable to all three of the most commonly prescribed PDE5 inhibitors and is likely related to nebivolol's ability to stimulate NO/cGMP in human erectile tissue regardless of diabetes status.

Considering that the prevalence of severe ED in diabetic men is almost three-fold higher than in the general population [26], and that ED in men with diabetes is more difficult to treat [27,28], the development of therapeutic strategies targeted to combat ED in diabetic men represents an outstanding challenge in the field. PDE5 inhibitors are the first-line therapy for ED with demonstrated efficacy [29]. However, efficacy rates for treating ED with most widely available PDE5 inhibitors, sildenafil, tadalafil and vardenafil, are significantly lower in diabetic populations [30–32]. Similar percentages of success in diabetic ED patients have been reported with the recently approved PDE5 inhibitor, avanafil [33]. Although different pathophysiological mechanisms could contribute, the reduced efficacy of PDE5 inhibitors in diabetes could be related to the profound impairment of NO/cGMP pathway observed in HCC and HPRA from diabetic patients with ED which is significantly exacerbated in these patients when compared to other ED patients [22] while defective NO/cGMP pathway has been related to failure of therapy with PDE5 inhibitors [34]. Since PDE5 inhibitors act by enhancing NO/cGMP pathway, a severe deterioration of this signaling pathway would compromise the activity of these drugs. This is consistent with the observed reduction of the capacity of sildenafil, tadalafil and vardenafil to relax arterial and trabecular penile smooth muscle in erectile tissues from diabetic men, as previously reported for sildenafil [22]. In fact, a reduction of the ability of PDE5 inhibitors to accumulate cGMP in

cavernosal tissue from diabetic patients with ED is here demonstrated, in accordance to that observed for sildenafil in penile tissue from diabetic rats [23].

In addition to selectively blocking β_1-adrenoceptors, nebivolol has the capacity to produce NO-mediated vasodilation in animal [35] and human [36,37] vasculatures, including HPRA [23]. Many studies demonstrate the ability of nebivolol to increase the production/availability of NO [38–41]. Since nebivolol stimulates the NO/cGMP pathway, and has no deleterious but potential beneficial effects on erectile function, as evidenced by its clinical profile on sexual function [13,14], we hypothesized that it could positively influence the activity of PDE5 inhibitors. Previously, we found that long-term administration of nebivolol significantly improved erectile function in diabetic rats, an improvement that was accompanied by an increase of systemic NO and penile cGMP and was not shared by the β_1-blocker, atenolol [23]. In this study, we now demonstrate that nebivolol significantly enhances the capacity of PDE5 inhibitors to relax human erectile tissue from diabetic patients with ED. This enhanced capacity is demonstrated in both HCC and HPRA, the two vascular structures that have to adequately relax and vasodilate, respectively, to allow penile erection. In addition, comparable potentiation is produced with the different PDE5 inhibitors, sildenafil, tadalafil and vardenafil, strongly suggesting that potentaiting effects by nebivolol are related to the pharmacological inhibition of PDE5 rather than to a specific drug effect. The potentiation of sildenafil-induced relaxation by nebivolol was previously reported in HCC and HPRA from non-diabetic patients [23] and it is here confirmed and extended to the other PDE5 inhibitors, tadalafil and vardenafil in HCC and HPRA from men with and without ED. However, our most important finding is the fact that nebivolol is able to reverse the impaired capacity of PDE5 inhibitors to cause relaxation of HCC and vasodilation of HPRA in erectile tissue from diabetic patients with ED, yielding responses that are not different to that observed in non-diabetic subjects without ED.

The way in which nebivolol potentiates the relaxant capacity of PDE5 inhibitors is likely mediated by activation of the NO/cGMP pathway since the treatment of HCC with nebivolol augments the ability of PDE5 inhibitors to accumulate cGMP in this tissue. Furthermore, the reduced efficacy of PDE5 inhibitors to accumulate cGMP in HCC from DMED is reversed after treating with nebivolol, resulting in cGMP levels not different from that produced by the PDE5 inhibitors in HCC from NEND. Thus, nebivolol activates a defective NO/cGMP pathway to enhance the efficacy of PDE5 inhibitors to potentiate this pathway and facilitate penile smooth muscle relaxation in diabetic erectile tissue.

On the other hand, vasodilation caused by nebivolol in HPRA was potentiated by the three PDE5 inhibitors at therapeutic concentrations. Although the strategy of potentiating PDE5 inhibitor vasodilatory capacity with nebivolol treatment seems more reasonable than the opposite based upon how both drugs are generally prescribed, these experiments reinforce the hypothesis that nebivolol acts through the NO/cGMP pathway and that a more efficacious vasodilation would be obtained with the combined administration than with the individual interventions even in the presence of diabetic ED.

DMED patients were older than NEND and had other vascular risk factors in addition to diabetes. Since this work was not intended to establish new paradigms in the pathophysiology of diabetes, we thought that it would be more relevant to evaluate the effects of nebivolol on the vasodilatory efficacy of PDE5 inhibitors in erectile tissues from a population of DMED representative of those attending a consultation for ED rather than obtaining a selected DMED population with only diabetes as a risk factor for ED. On the other hand, the use of healthy tissue provides the required reference to assess the degree of the improvement caused by nebivolol on the efficacy of PDE5 inhibitors to relax HCC and vasodilate HPRA in pathological conditions.

The mechanism by which nebivolol activate NO/cGMP pathway could include increase of NOS activity [20,40,42], reduction of asymmetric dimethylarginine levels [43], reduction of superoxide production [44,45] and endothelial β_2-adrenergic receptor or β_3-adrenergic receptor activation [40,42,46,47]. Any of these actions by nebivolol could relieve NO/cGMP impairment in erectile tissue associated with diabetic ED and require further investigation.

Conclusion

By activating the NO/cGMP pathway, nebivolol enhances the capacity of PDE5 inhibitors, sildenafil, tadalafil and vardenafil, to relax HCC

and vasodilate HPRA even in erectile tissue from diabetic patients with ED where an impairment of NO/cGMP pathway is manifested. This effect compensates for the reduced efficacy of these PDE5 inhibitors to relax penile smooth muscle in DMED. These results would support clinical data indicating that nebivolol has a favorable profile with respect to ED [13–15]. Although a drug-drug interaction study of nebivolol with sildenafil showed that effects on vital signs, pulse and blood pressure, were approximately the sum of the effects of sildenafil and nebivolol [48], the potential hypotensive effects of the combination should be considered. The present study points to the possible therapeutic potential of nebivolol use in combination with PDE5 inhibitors for the management of diabetic ED, a difficult-to-treat form of ED that often requires surgical intervention.

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Conflict of Interest: Dr. Harold M. Wright is an employee of the Forest Research Institute, Inc., a subsidiary of Forest Laboratories, Inc. (manufacturers of nebivolol [Bystolic®] in the United States).

Statement of Authorship

Category 1

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(c) Analysis and Interpretation of Data
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Category 2

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