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Enhanced thromboxane receptor mediated-responses and impaired endothelium-dependent relaxation in human corpus cavernosum from diabetic impotent men: Role of protein kinase C activity.

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Abbreviations: cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; HCC: human corpus cavernosum; PKC: protein kinase C.

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ABSTRACT

We have evaluated the influence of PKC activity on penile smooth muscle tone in tissues from diabetic and non-diabetic men with erectile dysfunction. Corpus cavernosum strips (HCC) were obtained from impotent diabetic and non-diabetic men at the time of penile prosthesis implantation and studied in organ chambers. Contractility responses to a prostanoid precursor, to prostanoids, and to the endothelium dependent vasodilator, acetylcholine, were studied. Arachidonic acid (AA; 100 μM) caused cyclooxygenase-dependent relaxation of HCC. This relaxation was impaired in diabetic tissues and normalized by blocking thromboxane receptors (TP-receptors) with SQ29548 (20 nM). Diabetes did not affect PGE_1-induced relaxation, but it reduced relaxation induced by the PGE_1 metabolite, PGE_0. This effect was related to an interaction of PGE_0 with TP-receptors. Diabetic tissues had reduced endothelium-dependent relaxation, which was partially improved by SQ29548 and completely normalized by the PKC inhibitor, GF109203X (1 μM). In HCC from non-diabetic patients, treatment with the PKC activator, phorbol 12,13-dibutyrate (PDBu; 0.3 μM), significantly attenuated endothelium-dependent relaxation, an effect prevented by co-administration of GF109203X. Tissues from diabetic patients had enhanced sensitivity to the contractile effects of the TP-receptor agonist, U46619 (EC_{50} 0.65±0.42 nM vs 6.01±2.28 nM; in diabetic vs non-diabetic patients). Inhibition of PKC with GF109203X (1 μM), prevented diabetes-induced hypersensitivity to U46619-induced contractions (EC_{50} 8.55±3.12 μM). Over activity of PKC in diabetes is responsible for enhanced contraction and reduced endothelium-dependent relaxation of HCC smooth muscle. Such alterations can result in erectile dysfunction.

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INTRODUCTION

Diabetic men are at higher risk for suffering from erectile dysfunction than the general population (Feldman et al., 1994; Martín-Morales et al., 2001). Erectile function depends on the relaxant capacity of penile smooth muscle, which is required for vasodilation and cavernosal expansion leading to blood accumulation and penile erection (Saenz de Tejada et al., 1991). Human penile smooth muscle tone is regulated by a tight balance between contractile and relaxant mediators. Alteration of the physiological mechanisms of tone regulation leading to a disbalance that favours contractile pathways and/or reduce relaxation could cause the inability to achieve an adequate erection.

Prostanoids participate in the regulation of penile smooth muscle tone. EP receptors mediate relaxation while the TP receptors mediate contraction in human corpus cavernosum tissue (Angulo et al., 2002). Prostaglandin E_1 (PGE_1) has been shown to produce trabecular smooth muscle relaxation and penile erection, and has been widely used as intracavernosal therapy for impotence (Porst, 1996). PGE_1 has a short half-life but it may be converted to prostaglandin E_0 (PGE_0), which is an active metabolite with similar properties to PGE_1 but with a longer half-life (Ney et al., 1991).

Prostanoid-driven pathways can be altered by diabetes. Indeed, excessive production of contractile prostanoids (Davi et al., 1997; Koltai et al., 1990) or enhanced contractile responses to prostanoids have been described (Hattori et al., 1999; McCarty, 1998).

Several molecular mechanisms have been proposed to be responsible for the vascular alterations associated to diabetes, including hyperactivation of

the protein kinase C (PKC). The activity of PKC is known to be elevated in diabetes (Koya and King, 1998) and treatment with PKC inhibitors has been shown to improve vascular function in diabetic animals (Ishii et al., 1996).

The aim of the present work was to characterize penile smooth muscle contractility in tissues from diabetic men with erectile dysfunction and evaluate the role of PKC in altered responses.

METHODS

Human corpus cavernosum tissues.

Human corpus cavernosum specimens, were obtained from impotent diabetic and non diabetic men at the time of penile prosthesis insertion after giving informed consent. Some specimens were also collected from organ donors. Protocols were approved by the local Ethics Committee. Tissues were maintained at 4-6ºC in M-400 solution (composition per 100 ml: manitol, 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 from extraction (Angulo et al., 2002).

Organ chamber studies.

Strips of corpus cavernosum tissue (3 x 3 x 7 mm) were immersed in 8 ml organ chambers containing physiological salt solution (PSS) of the following composition (mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 24.9, glucose 11, KH_2PO_4 1.2, EDTA 0.027 at 37º C continuously bubbled with 95% O_2/5% CO_2 mixture to maintain a pH of 7.4. Each tissue strip was incrementally stretched to optimal isometric tension, as determined by maximal contractile response to 1 µM phenylephrine (Azadzoi et al., 1992; Kim et al., 1991). Contractile responses were evaluated by adding increasing cumulative concentrations of compounds on unstimulated strips. For the relaxation studies, tissues were contracted with 0.5 - 3 µM phenylephrine and relaxation responses were evaluated by cumulative additions of compounds to the chambers.

Measurement of cyclic AMP in human corpus cavernosum tissue.

Corpus cavernosum strips were immersed in 8 ml organ chambers containing PSS, maintained at 37ºC and aerated with 5% CO_2/95% O_2, pH of 7.4. Each tissue strip was incrementally stretched to optimal isometric tension, as determined by maximal contractile response to 1 μM phenylephrine. Then each tissue was given 0.5 μM phenylephrine, and the phophodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX; 100 μM) and allowed to incubate for 15 minutes; after which time tissues were treated with drug or vehicle. Tissues were allowed to incubate for another 5 minutes then immediately frozen in liquid nitrogen and stored at -80ºC until extraction for cyclic nucleotide assay. Tissues were extracted by homogenization in 6% trichloroacetic acid followed by ether (H_2O-saturated) extraction and lyophilization. Cyclic AMP was determined by ELISA using a kit from Cayman Chemical Co. (Ann Arbor, MI).

Protein determinations.

Proteins were determined using the Bio-Rad Protein Assay Kit microtiter plate assay procedure (Bio-Rad, Hercules, CA) with bovine serum albumin as standard.

Drugs and Materials.

Arachidonic acid, indomethacin, phenylephrine, acetylcholine chloride, 9,11-dideoxy-9α,11α-epoxymethano $PGF_{2\alpha}$ (U46619), 3-isobutyl-1-methylxanthine (IBMX) and phorbol 12,13-dibutyrate (PDBu) were obtained from Sigma Chemical Co. (St. Louis, MO). Prostaglandin E_1 (alprostadil) was obtained from Pharmacia (Barcelona, Spain). Prostaglandin E_0 (13, 14 dehydro-PGE_1) was purchased from Cayman Chemical (Ann Arbor, MI). [1S-[1α,2α(Z),3α,4α]]-7-[3-

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[[2-[(Phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid (SQ29548) was obtained from Research Biochemical International (Natick, MA). Bisindoleyl-maleimide I (GF109203X; 3-[1-[3-(Dimethylamino)propyl]1H-indol-3-yl]-4-(1H-indol-3-yl)1H-pyrrole-2,5dione) was obtained from Alexis (Lausen, Switzerland). Prostanoid derivatives were dissolved at 10 mM concentration in ethanol and GF109203X was dissolved in dimethyl sulfoxide (DMSO) at 10 mM concentration (final concentration of DMSO was 0.0001%). Dilutions were made in distilled water at the time of the experiment. Non-prostanoid drugs were dissolved in distilled water. Indomethacin was dissolved in 1.5 mM Na_2CO_3.

Data analysis

Contractile effects produced by drugs are expressed as the percentage of maximal contraction to the agent or as the percentage of contraction elicited by 125 mM K^+ (KPSS, equimolar substitution of NaCl for KCl in PSS). Relaxation responses are expressed as percentage of total relaxation (loss in tone) induced by the addition of 0.1 mM papaverine HCl to the chambers at the end of the experiment. All data are expressed as mean $\pm$ standard error. Complete concentration-response curves were compared by a two-factors analysis of variance (ANOVA) test using StatView software for Apple computers. Cyclic AMP determinations were compared by a one-factor ANOVA followed by a Student-Newmann-Keuls test using GraphPad software for Apple computers.

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RESULTS

Specimens of corpus cavernosum tissue from 62 impotent patients (31 non diabetic and 31 diabetic patients) were obtained for this study. The demographic data and medical co-morbidities of these patients are shown in table 1. No significant differences in age or co-morbidities were observed between diabetic and non diabetic populations. Although the percentage of patients with arterial hypertension was higher among diabetic patients, this difference did not reach statistical significance (see Table 1). The data were insufficient to accurately differentiate non-insulin dependent diabetic (NIDD) men treated with insulin from those who were insulin dependent (IDD) patients and, of course, treated with insulin. However, endothelium-dependent relaxation was similar in HCC from diabetic patients treated with insulin or not (E_{max} for ACh: 50.0±5.8% in diabetic patients not receiving insulin, n=13, and 49.9%±3.73 in diabetic patients treated with insulin, n=18. Not significantly different).

Relaxation of human corpus cavernosum tissue induced by arachidonic acid.

Addition of arachidonic acid (AA; 100 μM) produced a relaxant response in human corpus cavernosum strips that was prevented by incubation with the cyclooxygenase inhibitor indomethacin (5 μM), in accordance with previous observations (Angulo et al., 2002). This relaxation was significantly impaired in tissues from diabetic patients. Treatment of diabetic tissues with the TP-receptor blocker, SQ29548 (20 nM) caused a full recovery of AA-induced relaxation (Fig. 1).

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Relaxant responses elicited by PGE_1 and PGE_0 in human corpus cavernosum tissue.

The active metabolite of PGE_1, PGE_0, relaxed corpus cavernosum tissue, but this relaxant response was reduced when compared to that induced by its parent molecule (Fig. 2A). PGE_0 (1 μM), however, produced an increase of cAMP content in human cavernosal tissue similar to that obtained with PGE_1 at the same concentration (Fig. 2B). The treatment of cavernosal tissue with SQ29548 (20 nM) did not modify relaxation induced by PGE_1 (data not shown), but significantly improved the relaxant responses evoked by PGE_0 (Fig. 2C). Indeed, treatment with SQ29548 abolished the differences between PGE_1- and PGE_0-induced relaxations (Fig. 2D).

Influence of diabetes on the relaxation of human corpus cavernosum to PGE_1 and PGE_0

The relaxations induced by PGE_1 in corpus cavernosum tissues from diabetic patients were not different from those obtained in tissues from non-diabetic patients (Fig. 3A). In contrast, the responses to PGE_0 were significantly impaired in tissues from diabetic patients (Fig. 3B). This impairment was reversed by treating the tissues with the TP receptor blocker, SQ29548 (20 nM) (Fig. 3C).

Effect of diabetes and TP-receptor blockade on endothelium dependent-relaxation of human corpus cavernosum.

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Human corpus cavernosum strips were relaxed by the cumulative addition of acetylcholine (ACh; 1 nM to 10 μM). The ACh-induced relaxation in this tissue was significantly impaired by diabetes. Treatment with SQ29548 (20 nM) did not alter endothelium-dependent relaxation of cavernosal strips from non diabetic patients, but significantly improved ACh-induced relaxation in corpus cavernosum from diabetic patients. However, SQ29548 was not able to completely recover endothelium-dependent relaxation in diabetic tissues (Fig. 4; in non diabetic patients, precontraction values were 2.40±0.74 and 2.43±1.01 for control and SQ29548, respectively; in diabetic patients, precontraction values were 2.14±0.67 g and 2.41±0.75 g for control and SQ29548, respectively. Not significant).

Effects of modulation of PKC activity on endothelium-dependent relaxation of corpus cavernosum from diabetic and non-diabetic patients.

The inhibition of PKC activity with GF109203X (1 μM) significantly potentiated ACh-induced relaxation of corpus cavernosum from diabetic patients (Fig. 5; in non diabetic patients, precontraction values were 3.44±0.46 and 3.05±0.68 for control and SQ29548, respectively; in diabetic patients, precontraction values were 3.00±0.91 g and 2.86±0.81 g for control and SQ29548, respectively. Not significant). After treating with GF109203X, endothelium-dependent relaxation of diabetic corpus cavernosum was not different from that of tissues from non diabetic patients. Conversely, the stimulation of PKC activity with a phorbol ester, PDBu (0.3 μM) drastically reduced endothelium-dependent relaxation to ACh in human corpus cavernosum from non diabetic patients (Fig 6A; precontraction values were

2.29±0.68 g and 1.68±0.41 g for control and PDBu, respectively. Not significant). This inhibitory effect of PDBu on ACh-induced relaxation was prevented by co-treating cavernosal strips with the PKC inhibitor, GF109203X (1 μM) (Fig. 6B; precontraction values were 2.60±1.02 g and 2.76±0.95 g for control and PDBu plus GF109203X, respectively. Not significant).

Effects of diabetes and PKC inhibition on the contraction of human corpus cavernosum mediated by TP receptors

The thromboxane analogue, U46619, produced concentration-dependent contractions of HCC smooth muscle. Tissues from diabetic patients showed enhanced sensitivity to U46619 compared to tissues from non-diabetic patients (Fig. 7A) as demonstrated by lower EC_{50} values for the TP receptor agonist (0.65±0.42 nM and 6.01±2.28 nM for diabetic and non-diabetic tissue, respectively, $p < 0.05$). Treatment with the PKC inhibitor, GF109203X (1 μM), did not alter U46619-induced contractions in corpus cavernosum tissue from non diabetic patients, but significantly inhibited these responses in cavernosal tissue from diabetic patients. In fact, treatment with GF109203X completely prevented the hypersensitive contractile responses to U46619 of diabetic tissues (Fig. 7A). Neither diabetes nor PKC inhibition significantly affected maximal contractile response to U46619 (Fig. 7B).

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DISCUSSION

The changes in tone following the addition of arachidonic acid (AA), which are prevented by indomethacin, show the capacity of human corpus cavernosum (HCC) to synthesize cyclooxygenase products that affect contractility of penile smooth muscle. In this tissue, arachidonic acid promotes the synthesis of relaxant and constrictor prostanoids. EP_2/EP_4-receptors and TP-receptors for relaxation and contraction, respectively, mediate responses to prostanoids in HCC (Angulo et al., 2002). The reduction of AA-induced relaxation in diabetic HCC could be due to an impairment of synthesis/activity of relaxant prostanoids or to an enhancement of synthesis/activity of contractile prostanoids. Our results favour the later explanation, since blockade of TP-receptors with SQ29548 normalised relaxation to AA in HCC from diabetic patients.

Although PGE_0 caused an increase of cAMP content in human cavernosal tissue similar to that induced by PGE_1 at the same concentration, indicating comparable capacity of the molecules to activate adenylyl cyclase, its capacity to relax HCC was reduced compared to that of its parental molecule. Since SQ29548 eliminated the differences between PGE_0 and PGE_1, it is likely that PGE_0, but not PGE_1, interacts with TP-receptors. Also, since diabetes impaired the relaxant capacity of PGE_0 in human trabecular tissue, but not PGE_1-induced relaxations and the blockade of TP receptors made responses to PGE_0 between diabetic and non diabetic tissues comparable, an increased TP receptor-mediated response seems to be responsible for diabetes-induced reduction of relaxation to PGE_0. Diabetes-induced alteration

of the TP-receptor mediated pathway is demonstrated by the hypersensitivity to the contractile activity of the thromboxane analogue, U46619 observed in diabetic tissues. Furthermore, hypersensitivity of TP receptor-mediated responses may also be involved in the impairment of endothelium-dependent relaxation of HCC associated to diabetes, since blockade of these receptors partially improved ACh-induced relaxation in HCC from diabetic men.

Vascular smooth muscle contraction induced by ligands of TP receptor involves G protein-mediated activation of phospholipase C (PLC) which promotes inositol tri-phosphate (IP_3) generation and subsequent release of calcium from intracellular stores, leading to the activation of contractile machinery (Hirata et al., 1991; Coleman et al., 1994). The activity of PLC also yields diacylglycerol (DAG) which is an activator of PKC. In addition, the increase of intracellular calcium concentration could facilitate the activation of some PKC isoforms. Activated PKC could activate Ca^{2+}-channels that would contribute to increases in intracellular calcium concentration, participating in agonist-induced contraction (Fish et al., 1988; Navedo et al., 2005). PKC activity could also potentiate contraction by enhancing calcium-sensitivity of contractile mechanisms (Gokina et al., 1999; Ding and Murray, 2005) an effect also demonstrated in human arteries (Martínez et al., 2000). In fact, PKC participates in calcium sensitizing pathways of contraction of rabbit corpus cavernosum smooth muscle (Takahashi et al., 2003).

Despite the above mentioned findings, the relevance of PKC activation in physiologic contraction mediated by TP receptors remains controversial. In rat mesenteric artery, PKC inhibitors have been shown to significantly reduce U46619-mediated contractions (Tasaki et al., 2003) while no alteration in

mesenteric artery contraction to U46619 by PKC inhibitors has also been reported in *in vivo* studies (Bauer et al., 1999). In addition, inhibition of PKC did not modify U46619-mediated contraction of pulmonary circulation in rats and cats (Kaye et al., 1995). Consistent with these findings, we find in this study that contraction of HCC from non-diabetic patients to U46619 was not influenced by PKC inhibition. This suggests that the cellular signalling pathway triggered by TP receptor activation in HCC, at least in non-diabetic conditions, does not involve the participation of PKC.

Diabetes is associated with an increase in PKC activity in vascular tissue, probably related to increased glucose-induced *de novo* formation of DAG. Increase of PKC activity under hyperglycemic conditions has been previously observed in cultured human cavernosal cells (Ganz and Seftel, 2000). Hypersensitivity to TP receptor activation in diabetic HCC is likely mediated by a PKC-dependent mechanism, since inhibition of PKC completely reversed the hypersensitive response to U46619. The observation that PKC inhibition reverses diabetes-induced potentiation of TP receptor-mediated contraction and that TP receptor blockade improves endothelium-dependent relaxation in diabetic HCC, would suggest that the beneficial effects of PKC inhibition on endothelial function in HCC from diabetic patients could be attributed to its influence on TP-mediated responses. But, while the improvement in endothelium-dependent relaxation by TP-receptor blockade was only partial, PKC inhibition completely reversed endothelial dysfunction, suggesting that PKC overactivity is influencing other components of endothelium-dependent relaxation in diabetic tissues. The specific PKC isoform involved cannot be determined in our study, since GF109203X, at the

concentration used in this study, has been shown to inhibit the PKC isoforms, α, βI, βII, γ, δ and ε (Toullec D et al., 1991; Martiny-Baron G et al., 1993).

Overactivity of PKC could impair endothelial function through different pathophysiological mechanisms. PKC activation increases NADPH oxidase activity (Gorlach et al., 2000) and induces the uncoupling eNOS activity (Hink et al., 2001). These actions lead to generation of superoxide anion which reduces the bioavailability of NO for causing endothelial relaxation. In addition, PKC activity has also been suggested to inhibit post-translational activation of eNOS (Michell et al., 2001), compromising NO production. Thus, the overactivity of PKC associated to diabetes could, in addition to potentiate TP receptor-mediated responses, inhibit endothelium-dependent relaxation by affecting the NO-mediated responses.

In our study, further support for the pathophysiologic role of excessive activity of PKC, was demonstrated by provoking impairment of endothelium-dependent-relaxation by inducing overactivity of PKC with a phorbol ester in HCC from non-diabetic patients. Nangle and collaborators previously reported an improvement of endothelium-dependent and neurogenic relaxations of corpus cavernosum from diabetic mice after chronic PKC inhibition (Nangle et al., 2003).

We demonstrate that diabetes causes hypersensitivity to the contractile effects of prostanoids in HCC by a mechanism involving overactivity of PKC. This overactivity of PKC is also involved in the impairment of endothelium-dependent relaxation in tissue from diabetic impotent men. Thus TP receptor blockade and PKC inhibition are therapeutic targets for the treatment of ED associated with diabetes.

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Figure Legends

Figure 1. Effects of diabetes and the treatment with indomethacin (5 μM) or SQ29548 (20 nM) on loss in tone induced by the addition of arachidonic acid (AA; 100 μM) in human trabecular smooth muscle strips contracted with phenylephrine. Data are expressed as mean±SEM of the percentage of total relaxation induced by 0.1 mM papaverine. n indicates the number of patients from whom the tissues were collected for the experiments. *** indicates $p < 0.001$ vs no diabetes by a two-factors ANOVA test.

Figure 2. Panel A shows the responses elicited by prostaglandin E_1 (PGE_1; 1 nM to 10 μM) and prostaglandin E_0 (PGE_0; 1 nM to 10 μM) on human trabecular smooth muscle strips contracted with phenylephrine. Panel B shows the cAMP tissue content of human corpus cavernosum after exposure to PGE_1 (1 μM) or PGE_0 (1 μM). Panel C shows the effects of the treatment with the TP receptor antagonist, SQ29548 (20 nM), on the relaxations elicited by PGE_0 while in panel D relaxations to PGE_1 and PGE_0 in the presence of SQ29548 are compared. Data are expressed as mean±SEM of the percentage of total relaxation induced by 0.1 mM papaverine in A, C and D. Data are expressed as mean ± SEM of pmol cAMP per mg of tissue protein content in B. n indicates the number of patients from whom the tissues were collected for the experiments. *** indicates $p < 0.001$ vs PGE_1-induced responses in A and vs control in C by a two-factors ANOVA test. * $p < 0.05$ vs control in B by a Student-Newmann-Keuls post-hoc test.

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Figure 3. Effects of diabetes on the relaxations elicited by prostaglandin E_1 (PGE_1; 1 nM to 10 μM) (A) and on the relaxations induced by prostaglandin E_0 (PGE_0; 1 nM to 10 μM) in the absence (B) or the presence (C) of the TP receptor antagonist, SQ29548 (20 nM) in human trabecular smooth muscle strips contracted with phenylephrine. Data are expressed as mean±SEM of the percentage of total relaxation induced by 0.1 mM papaverine. n indicates the number of patients from whom the tissues were collected for the experiments. *** indicates $p < 0.001$ vs responses in strips from non diabetic patients by a two-factors ANOVA test.

Figure 4. Effects of diabetes and blockade of TP-receptors with SQ29548 (20 nM) on endothelium dependent relaxation of human corpus cavernosum strips contracted with phenylephrine. Data are expressed as mean±SEM of the percentage of total relaxation induced by 0.1 mM papaverine. n indicates the number of patients from whom the tissues were collected for the experiments. *** indicates $p < 0.001$ vs no diabetes and †† indicates $p < 0.01$ vs diabetes by a two-factors ANOVA test.

Figure 5. Effects of diabetes and the treatment with the PKC inhibitor, GF 109203X (1 μM) on endothelium dependent relaxation of human corpus cavernosum strips contracted with phenylephrine. Data are expressed as mean±SEM of the percentage of total relaxation induced by 0.1 mM papaverine. n indicates the number of patients from whom the tissues were collected for the

experiments. *** indicates $p < 0.001$ vs no diabetes and ††† indicates $p < 0.01$ vs diabetes by a two-factors ANOVA test.

Figure 6. Effects of PKC activation with phorbol 12,13-dibutyrate (PDBu) (0.3 μM) on endothelium dependent relaxation of human corpus cavernosum strips contracted with phenylephrine in the absence (A) or the presence of the PKC inhibitor GF109203X (1 μM) (B). Data are expressed as mean±SEM of the percentage of total relaxation induced by 0.1 mM papaverine. n indicates the number of patients from whom the tissues were collected for the experiments. *** indicates $p < 0.001$ vs control by a two-factors ANOVA test.

Figure 7. Effects of diabetes and the treatment with the PKC inhibitor, GF 109203X (1 μM) on the contractile responses elicited by the agonist of TP receptors, U46619 (0.01 nM to 3 μM), in human trabecular smooth muscle strips. In panel A, data are expressed as mean±SEM of the percentage of maximal contraction elicited by U46619 in each case. In panel B, maximal contraction to U46619 for every treatment is expressed as mean±SEM of the percentage of contraction induced by 125 mM K^+ (KPSS). n indicates the number of patients from whom the tissues were collected for the experiments. *** indicates $p < 0.001$ vs responses in strips from non diabetic patients by a two-factors ANOVA test.

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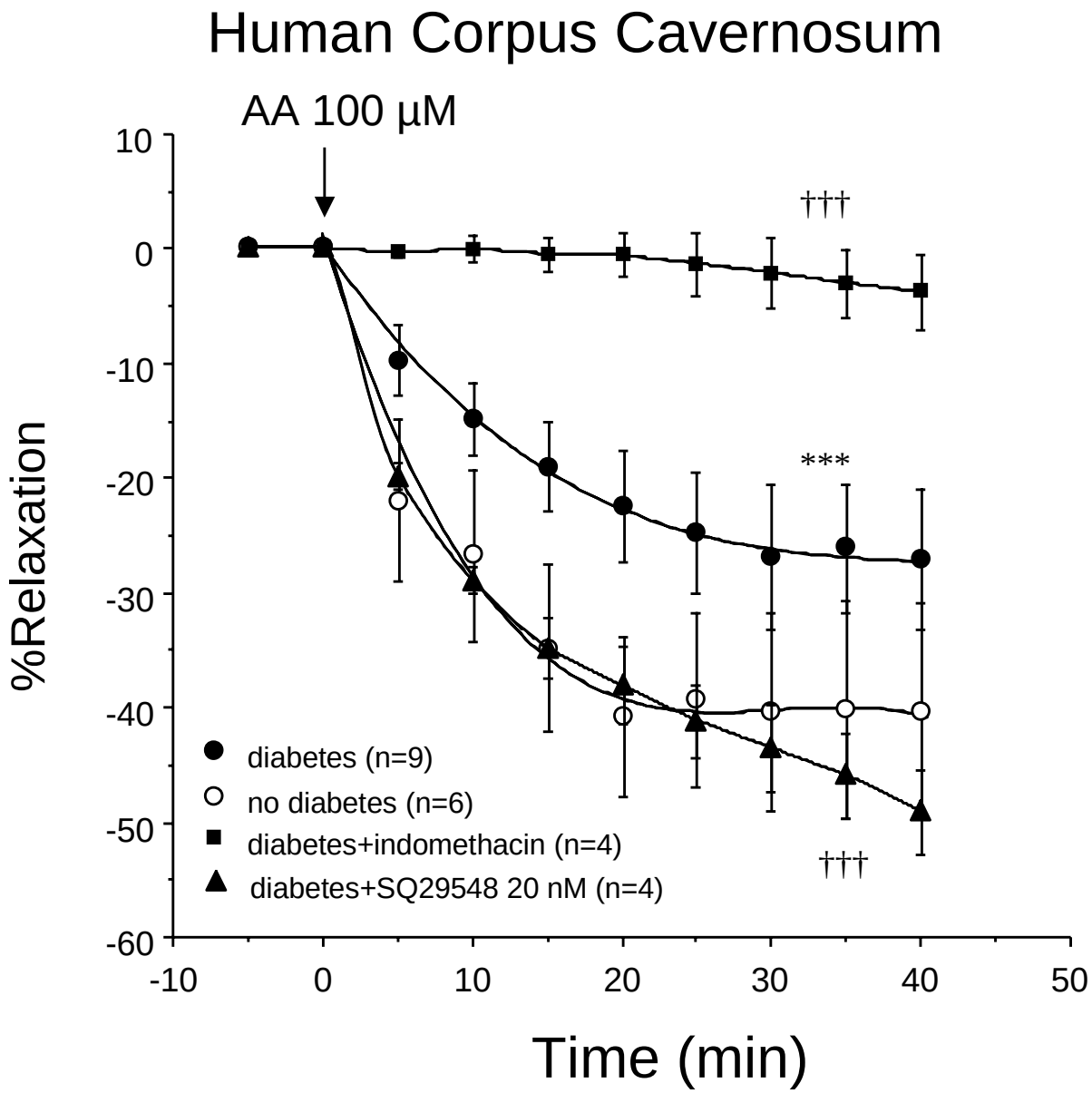
Table 1. Demographic data and co-morbidities of patients from whom the tissues were collected for the study

	Non diabetic patients	Diabetic patients
n	31	31
Age (years)	58.2±1.6	59.5±1.4
Treatment for diabetes Hypoglycemiants Insulin Diet-control		 9 (29.0%) 18 (58.1%) 4 (12.9%)
Hypercholesterolemia	7 (22.6%)	7 (22.6%)
Hypertension	5 (16.1%)	10 (32.2%)
Smoking habit	21 (67.7%)	18 (58.1%)
Neurological alterations	6 (19.3%)	8 (25.8%)
Hormonal alterations	1 (3.2%)	2 (6.4%)

Figure 1



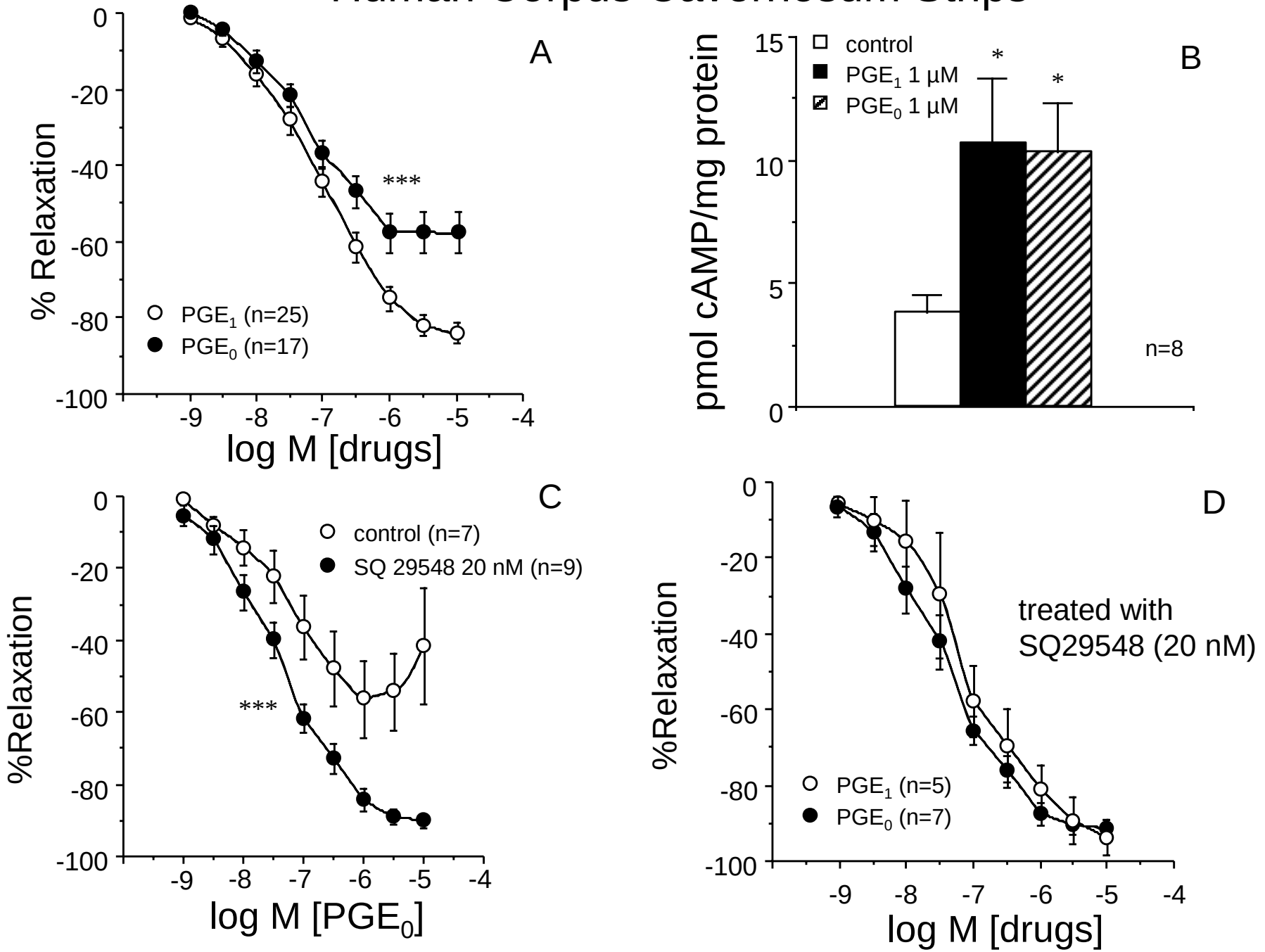


Figure 3

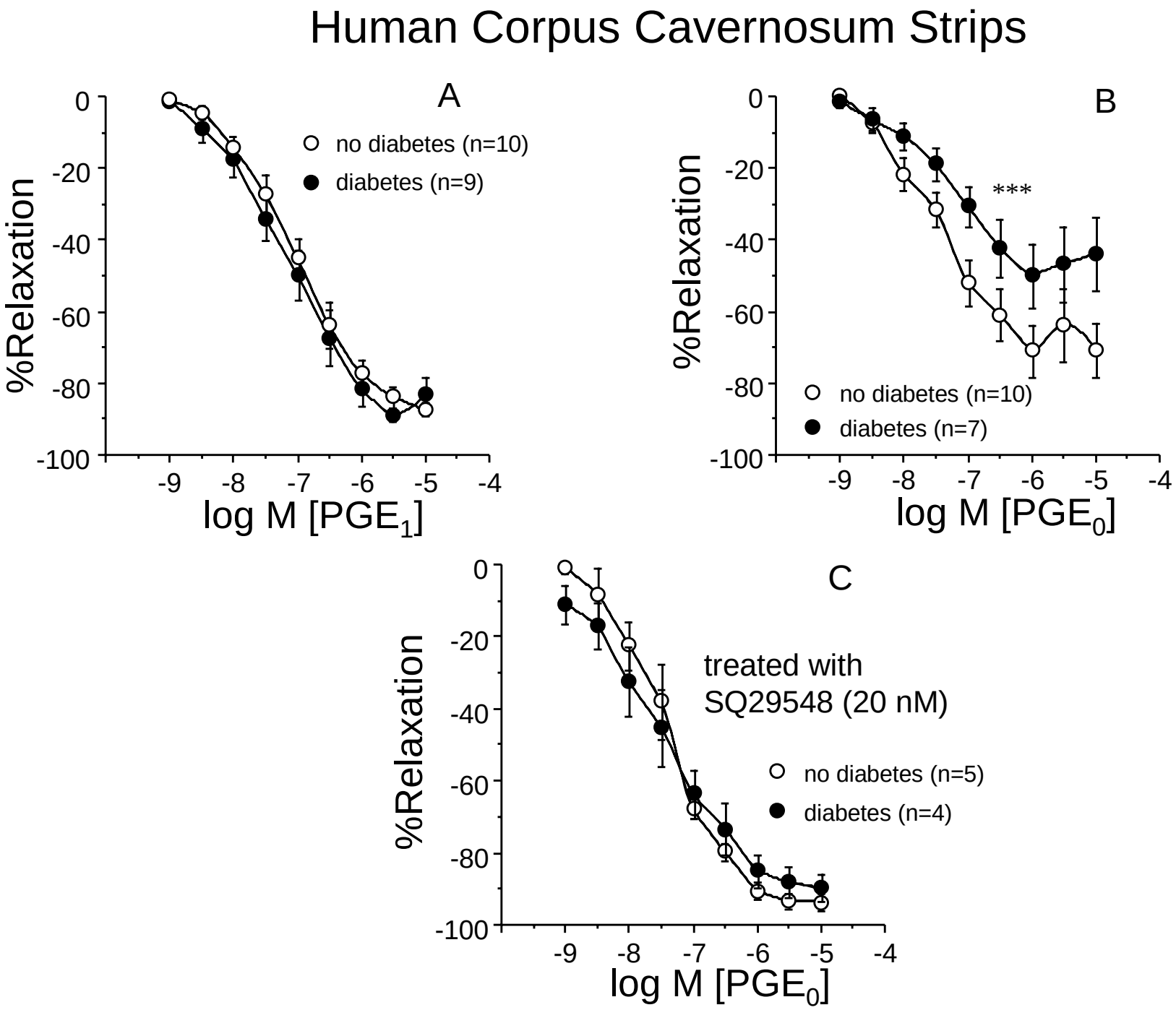


Figure 4

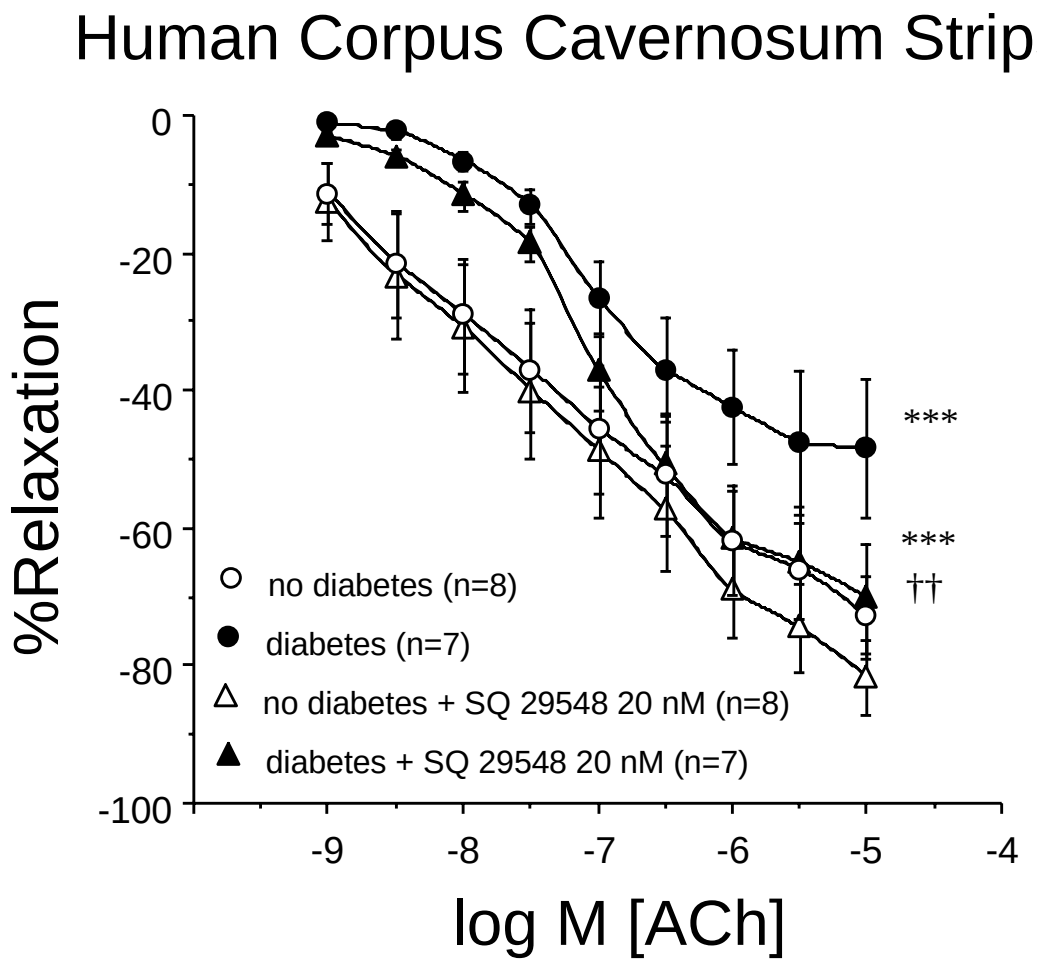


Figure 5

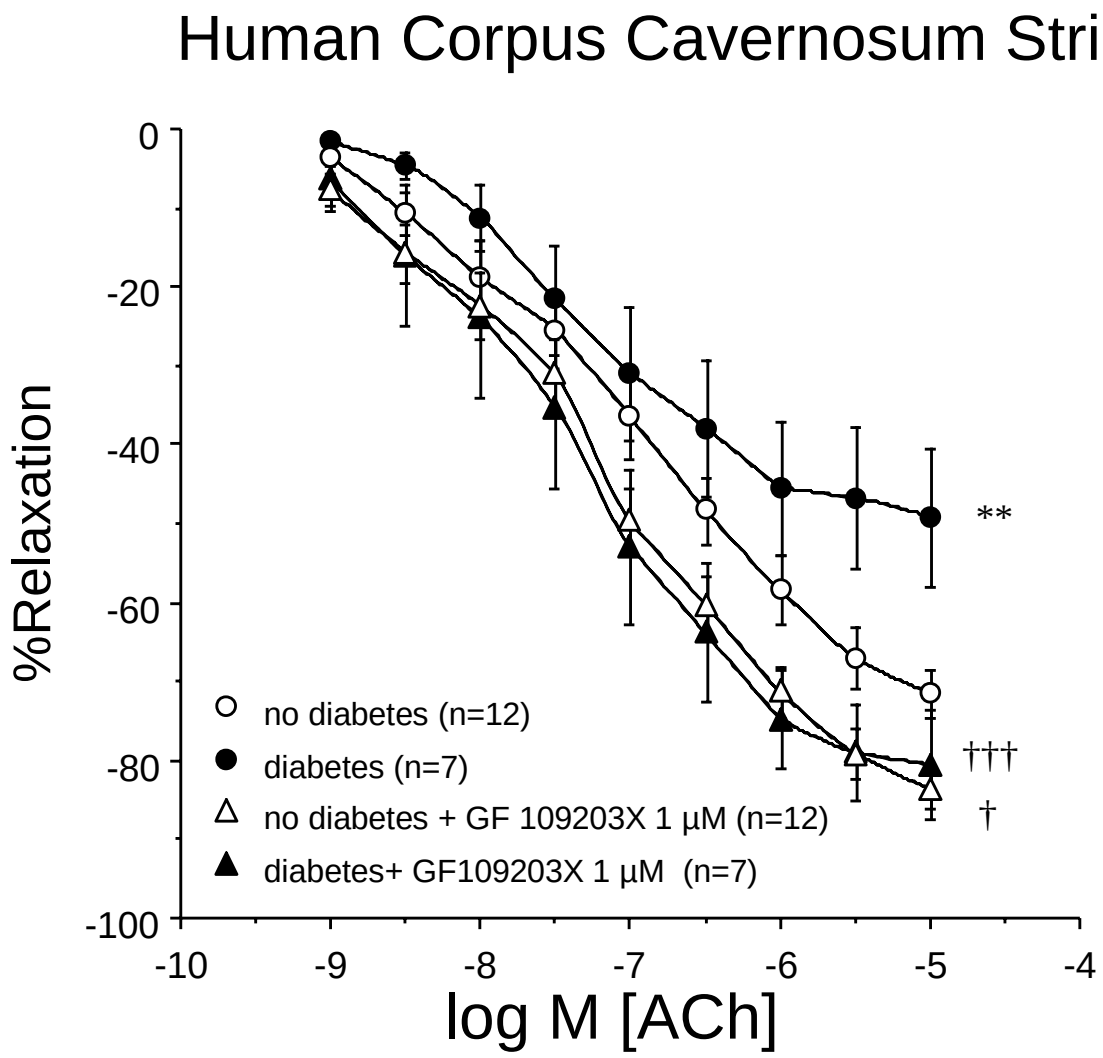


Figure 6

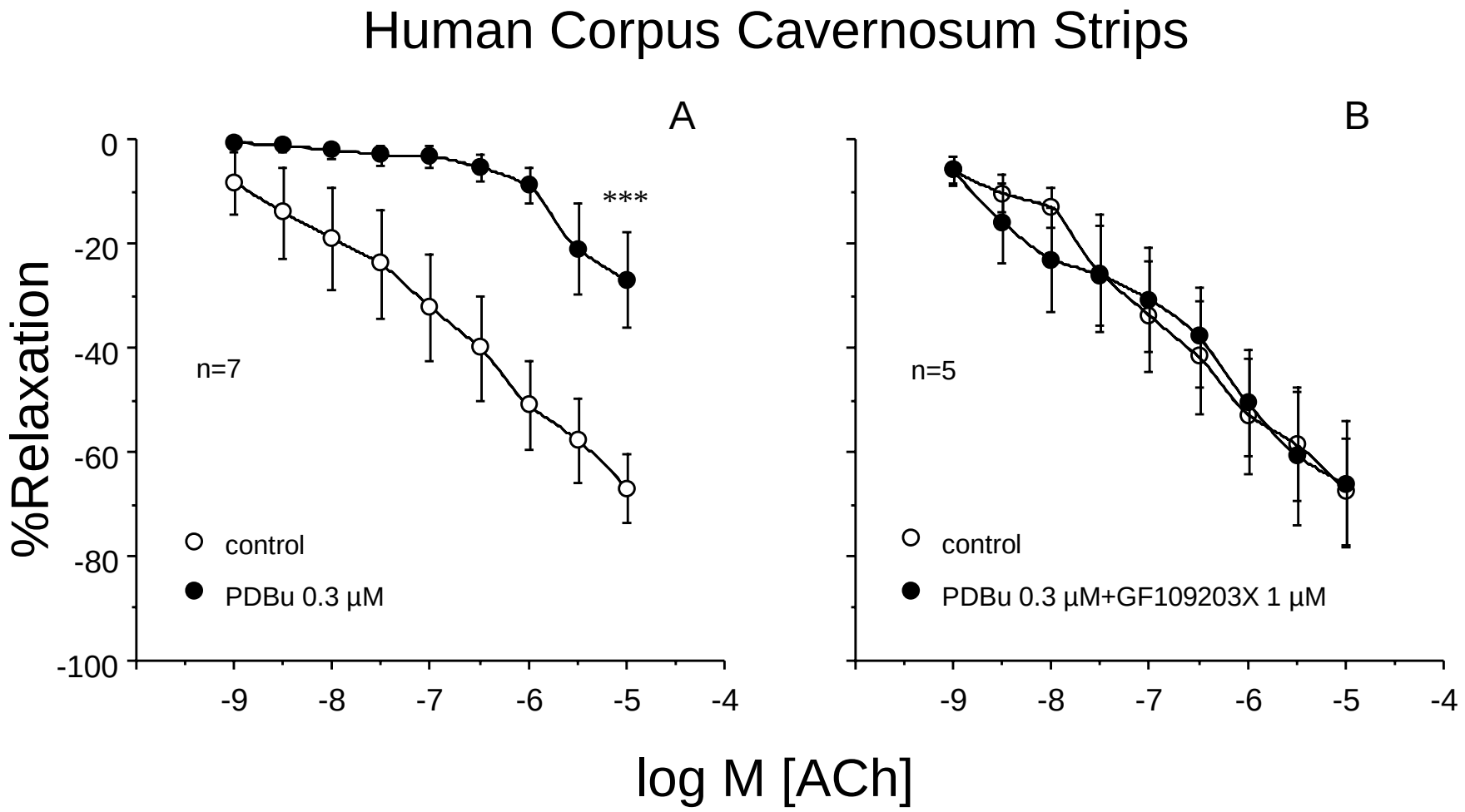


Figure 7

Human Corpus Cavernosum Strips