

**Type 1 diabetes induced hyperresponsiveness to 5-HT in rat pulmonary arteries
via oxidative stress and induction of cyclooxygenase-2¹**

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List of nonstandard abbreviations: 5-HT, serotonin; BMPR2, bone morphogenetic protein receptor type 2; BW, body weight; COX-2, cyclooxygenase-2; E_{max} maximal contractile effect; K_v, voltage-gated potassium channels; LV, left ventricle; PA, pulmonary artery; PAH, pulmonary arterial hypertension; S, septum; SAP, systemic arterial pressures; RV, Right ventricle; RVSP, Right ventricular systolic pressure, SOD, superoxide dismutase; TXA₂, thromboxane A₂.

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Abstract

Recent epidemiological data suggest that diabetes is a risk factor for pulmonary arterial hypertension. The aim of the present study was to analyze the link between type 1 diabetes and pulmonary arterial dysfunction in rats. Male Sprague-Dawley rats were randomly divided into a control (saline) and a diabetic group (70 mg/kg streptozotocin). After 6 weeks, diabetic animals showed a downregulation of the lung bone morphogenetic protein receptor type 2 (BMPR2), upregulation of 5-HT_{2A} receptors and cyclooxygenase-2 proteins as measured by Western blot analysis and increased contractile responses to 5-HT in isolated intrapulmonary arteries. The hyperresponsiveness to 5-HT was endothelium-independent and unaffected by inhibition of NO synthase but prevented by indomethacin, the selective cyclooxygenase-2 inhibitor NS398, superoxide dismutase and the NADPH oxidase inhibitor apocynin or chronic treatment with insulin. However, diabetic rats at 6 weeks did not develop elevated right ventricular pressure or pulmonary artery muscularization while a longer exposure (4 months) to diabetes induced a modest but significant increase in right ventricular systolic pressure. In conclusion, type 1 diabetes mellitus in rats induces a number of changes in lung protein expression and pulmonary vascular reactivity characteristic of clinical and experimental pulmonary arterial hypertension but insufficient to elevate pulmonary pressure. Our results further strengthen the link between diabetes and pulmonary arterial hypertension.

Introduction

Despite the fact that type 1 and type 2 diabetes are strongly associated with systemic cardiovascular diseases (Rutter et al., 2005) the relationship with pulmonary vascular disease has been almost disregarded (Fouty, 2008). However, recent epidemiological studies suggest a link between diabetes and pulmonary arterial hypertension (PAH) (Movahed et al., 2005; Makarevich et al., 2007; Zamanian et al., 2009). In addition, maternal diabetes is an independent risk factor for persistent pulmonary hypertension of the newborn (Hernandez-Diaz et al., 2007). There is also some experimental evidence linking type 2 diabetes with PAH. Thus, male apoE^{-/-} mice on a high-fat diet, an animal model associated to insulin resistance, develop PAH which was prevented by the antidiabetic drug rosiglitazone (Hansmann et al., 2007).

PAH exhibits a complex pathophysiology, unlikely to be explained by a single factor (Chan and Loscalzo, 2008; Rabinovitch, 2008). Mutations in the bone morphogenetic protein receptor type 2 (BMPR2) underlie many heritable and sporadic cases of PAH (Lane et al., 2000) and downregulation of its expression is a common feature of several forms of PAH (Atkinson et al., 2002). Inactivation, downregulation or gene polymorphisms of voltage-gated potassium channels (K_v) have also been implicated in PAH (Yuan et al., 1998). Several lines of evidence also indicate that serotonin (5-hydroxytryptamine [5-HT]) plays a central role in the pathogenesis of this entity. Thus, 5-HT stimulates pulmonary artery (PA) contraction and smooth muscle cell proliferation and blocks K_v channels (MacLean et al., 2000; Cogolludo et al., 2006b). Hyperresponsiveness to 5-HT in large and small pulmonary arteries (PA) is a common feature of PAH (Le Cras et al., 2000; Sato et al., 2000; Keegan et al., 2001; Thomas and

Wanstall, 2003; Rodat et al., 2007). Elevated plasma levels of 5-HT and overexpression or polymorphisms in the genes encoding 5-HT receptors or the 5-HT transporter are associated with the disease (MacLean et al., 2000). It has also been suggested that local endothelium-derived 5-HT acting in a paracrine manner may be involved in PAH (Eddahibi et al., 2006). Furthermore, pharmacological inhibition or genetic deletion of 5-HT receptors or the 5-HT transporter attenuates PAH and prolongs survival (MacLean et al., 2000). Loss of NO bioavailability is an additional component of the endothelial dysfunction and vascular pathology found in PAH (Coggins and Bloch, 2007).

In rats treated with streptozotocin, a widely used model of type 1 diabetes, we have recently found pulmonary endothelial dysfunction associated to increased superoxide production and upregulation of the NADPH oxidase subunit p47^{phox} (Lopez-Lopez et al., 2008). Right ventricular hypertrophy has also been found in this model (Al-Shafei et al., 2002). We hypothesized that type 1 diabetes could lead to the development of PAH. Therefore, the present study was designed to analyze the effects of streptozotocin on pulmonary markers of PAH including the pulmonary expression of key proteins of the disease, K_v currents, PA pressure and right ventricular hypertrophy. We also further analyzed the mechanisms contributing to the vascular hyperreactivity of PA to 5-HT.

Methods

Animals and treatments

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 85-23, revised 1996), and the procedures

were approved by our institutional review board. Male Sprague-Dawley rats were randomly divided into a control and a diabetic group. Diabetes was induced by a single intraperitoneal injection of 70 mg/kg streptozotocin (controls were injected with saline) and followed for 6 weeks (except a group which was followed for 4 months). In a further experimental series, streptozotocin treated rats were randomly co-treated with insulin glargine (5 units/Kg, once daily) or saline. Blood glucose was analyzed using a clinical glucometer.

Pressure measurements

Systolic and diastolic systemic arterial pressures (SAP) were analyzed with a pressure transducer via a catheter located in the right carotid artery in anesthetized (pentobarbitone 30-50 mg/kg i.p.) rats ventilated with room air. Right ventricular systolic pressure (RVSP) was then measured in open chest rats with a pressure transducer via a catheter advanced through the right jugular vein and placed into the right ventricle.

Lung histology

The right lung was inflated in situ with formol (via a column of 25 cm of height through the trachea) and embedded in paraffin. Lung sections were stained by hematoxylin/eosin and Masson trichrome techniques and examined by light microscopy. Elastin was visualized by its green autofluorescence. Small arteries were analyzed in a blinded fashion and categorized as muscular, partially muscular, or nonmuscular.

Vascular reactivity

Intrapulmonary artery rings (2-3 mm long, internal diameter ~0.5-0.8 mm) were dissected and mounted in Krebs solution under 0.75 g of resting tension in organ chambers as previously described (Cogolludo et al., 2006b). In some experiments, the endothelium was removed by gently rubbing the intimal surface of the rings with a metal rod. The endothelium removal procedure was verified by the inability of acetylcholine (10^{-6} M) to relax arteries precontracted with 10^{-6} M phenylephrine. After equilibration, rings were precontracted by 80 mM KCl, and, once a stable contraction was reached, were washed with Krebs solution for 30 minutes and concentration-response curves to 5-HT (10^{-8} - 10^{-4} M) were performed by cumulative addition in the absence or presence of drugs.

K_v current recordings

PA smooth muscle cells were enzymatically isolated and membrane currents were recorded using the whole-cell configuration of the patch clamp technique as previously described (Cogolludo et al., 2006b).

Western blot analysis

Pulmonary artery or whole lung homogenates were run on a SDS-PAGE, and Western blot was performed as described (Moreno et al., 2004) using primary monoclonal mouse anti- α - or anti- β -actin (Sigma), anti-BMP2 (BD Transduction), anti-5HT_{2A} (Pharmingen), anti-COX-2 (Cayman) or anti-K_v1.5 (Alomone) antibodies.

Statistical analysis

Results are expressed as means \pm SEM of measurements. Statistical analysis was performed by an unpaired Student's *t*-test and for multiple comparisons by one-way

ANOVA followed by a Newman Keuls's test. $P < 0.05$ was considered statistically significant. Individual cumulative concentration-response curves were fitted to a logistic equation. The maximal drug effect (E_{max}) and the drug concentration producing 50% of the E_{max} (EC_{50}) expressed as negative log molar value (pD_2) were calculated from the fitted curves for each ring. Apparent pK_B were calculated according to the equation $pK_B = \log(DR-1) - \log[B]$, where DR is the ratio of the mean EC_{50} values of the agonist in the presence and in the absence of a given concentration ($[B]$) of the antagonist.

Results

Right ventricular systolic pressure and right ventricular weight

After 6 weeks of streptozotocin treatment, animals developed the expected increase in blood glucose (488 ± 39 mg/dL, $P < 0.01$ vs 134 ± 13 mg/dL in control animals) and decrease in body weight (196 ± 13 g, $P < 0.01$ vs 367 ± 10 g in control animals). We found no significant changes in RVSP or in the ratios of the free wall of the right ventricle (RV) to body weight (BW), free wall of the left ventricle (LV) plus septum (S) to BW or $RV/(LV+S)$ as compared with controls (Figure 1A). Moreover, diabetes did not modify the percentage of muscular, partially muscular or non muscular arteries (Figure 1B). However, in the group treated for four months with streptozotocin (blood glucose 473 ± 31 mg/dL, $P < 0.01$ vs 125 ± 5 mg/dL in control animals) there was a significant increase in RVSP compared to parallel controls (22.4 ± 1.9 , $n = 6$ vs 16.7 ± 0.7 mm Hg, $n = 4$, respectively, $P < 0.05$).

Lung BMPR2 but not $K_V1.5$ expression is downregulated in diabetes

Since BMPR2 and $K_v1.5$ are key proteins involved in PAH we examined its expression in diabetic lungs. Diabetes induced a clear downregulation of lung BMPR2 expression (Figure 2A). In contrast, the expression of $K_v1.5$ channel protein was not significantly modified in these diabetic animals (Figure 2B). In isolated PA smooth muscle cells, membrane capacitance, an estimate of membrane surface, was similar in both groups (14 ± 2 pF and 14 ± 1 pF in control and diabetic, respectively, not significant). We also observed a non significant decrease in the amplitude of the K_v currents in diabetic animals (Figure 2C).

PA from diabetic rats show endothelium-independent hyperresponsiveness to 5-HT

5-HT induced a concentration-dependent contractile response which was higher in diabetic compared to control rats (Figure 3A). The analysis of the concentration-response curve indicated that diabetes induced an increase in the maximal contractile response (E_{max} , Figure 3C) without significant changes in the concentration of 5-HT required for half-maximal contraction (pD_2 , Figure 3C). Because this increased response could be attributed to a reduced NO bioavailability (Lopez-Lopez et al., 2008) similar experiments were carried out in the presence of the NOS inhibitor L-NAME. This drug increased the maximal response to 5-HT in both diabetic and control animals ($p < 0.05$) but the difference between both groups remained highly significant ($P < 0.01$, Figure 3B and 3C). Similarly, in endothelium-denuded arteries the response to 5-HT was higher ($P < 0.01$) than in intact ones but again diabetic denuded vessels were strongly hyperresponsive ($P < 0.01$, Figure 3C and 4A). In endothelium-denuded arteries from rats co-treated with streptozotocin plus insulin (blood glucose = 178 ± 5 mg/dL) the

E_{\max} of 5-HT was reduced ($115 \pm 17\%$, $P < 0.05$) when compared to the parallel group of streptozotocin-treated rats (glucose = 440 ± 26 , $E_{\max} = 201 \pm 17\%$).

Role of 5-HT_{2A} receptors

We analyzed the contractile response of 5-HT in endothelium-intact and denuded PA in the presence of the competitive antagonist of 5-HT_{2A} receptors ketanserin. This drug produced a rightward shift of the concentration-response to 5-HT (Figure 4A). The calculated pK_B values of ketanserin from this shift, an indicator of the potency of the antagonist, were 8.2 in both intact and denuded arteries (Figure 4B). These pK_B values are in agreement with the expected potency of the antagonist for 5-HT_{2A} receptors (8.1-9.7) (Alexander et al., 2008) indicating that these receptors appear to play a major role in 5-HT contraction as previously reported (MacLean et al., 1996). The potency of ketanserin was similar in control and diabetic animals in both intact and denuded vessels. To analyze the possible role of changes in 5-HT_{2A} receptors, its expression was analyzed in lung homogenates by Western blot. The expression of the 5-HT_{2A} receptors was increased ~ 2 fold in diabetic rats (Figure 4C).

Role of cyclooxygenase-2 (COX-2)

To analyze a possible role of COX, we tested the effects of the non selective COX inhibitor indomethacin in endothelium-intact vessels. This drug produced a weak rightward shift of the curve to 5-HT in PA from control rats (Figure 5A) leading to a significant decrease in the pD_2 value (Figure 5C). Interestingly, indomethacin abolished the hyperresponsiveness to 5-HT in diabetic rats. In the presence of the selective COX-2 inhibitor NS-398 (N-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide) the responses to 5-HT in control rats were similar to those in its absence. However, in

diabetic rats, this drug prevented the enhanced response to 5-HT and also induced a weak rightward shift of the curve (Figure 5B) leading to a significant decrease in the pD_2 value (Figure 5C). Consistent with a role for COX-2 in the vascular responses, the amount of this protein was strongly increased in PA from diabetic rats (Figure 5D).

Role of superoxide

As we have previously reported an increase in NADPH oxidase-derived superoxide in PA from diabetic rats (Lopez-Lopez et al., 2008), we analyzed the contractile response to 5-HT in endothelium-intact PA treated with superoxide dismutase (SOD) or the NADPH oxidase inhibitor apocynin. The contraction induced by 5-HT in control rats (Figures 6A and 6B) was similar to that observed in the absence of the drugs (Figure 3A). However, both treatments prevented the enhanced response to 5-HT in diabetic rats.

Effects of exogenous addition of superoxide and a thromboxane A_2 (TXA₂) analog

The responses to 5-HT in PA from control rats were analyzed in the presence of the superoxide generating drug pyrogallol or the TXA₂ analog U46619 ((Z)-7-[(1S,3S,4S)-3-[(E,3S)-3-hydroxyoct-1-enyl]-5-oxabicyclo[2.2.1]heptan-2-yl]hept-5-enoic acid). After washing the initial response to KCl, pyrogallol or U46619 were added at concentrations titrated to produce 5-15% of the response to KCl ($3 \times 10^{-6}M$ and $10^{-8}M$, respectively) and after 15 min the concentration-response to 5-HT was performed. Pyrogallol increased the E_{max} to 5-HT without changing the pD_2 value and this effect was prevented by indomethacin (Figure 7A), mimicking the results in diabetic rats. On the other hand, U46619 produced a leftward shift of the curve to 5-HT (increase in pD_2)

with a non significant increase in the E_{max} and this effect was not prevented by apocynin (Figure 7B).

Discussion

Despite the well known link between diabetes and systemic cardiovascular disease, the relationship with pulmonary vascular disease has been largely overlooked. In a previous study, insulin resistance in ApoE^{-/-} mice was associated to PAH (Hansmann et al., 2007). We previously found PA endothelial dysfunction, a characteristic feature of PAH, in rats with type 1 diabetes and right ventricular hypertrophy was also reported in streptozotocin treated rats (Al-Shafei et al., 2002; Lopez-Lopez et al., 2008). Herein, we show that rats treated with streptozotocin share a number of pulmonary vascular abnormalities with animal models and patients with PAH such as downregulation of BMPR2, COX-2 induction, upregulation of 5-HT_{2A} receptors and vascular hyperresponsiveness to 5-HT in addition to those previously described such as endothelial dysfunction and pulmonary vascular oxidative stress. RVSP and RV/(LV+S) and PA muscularization were not significantly different in diabetic rats compared to non diabetic controls at 6 weeks while at 4 months we found a significant increase in RVSP. This increase was modest, much lower than classical models of PAH.

Both clinical and experimental forms of PAH are associated with a decrease in K_v currents and diminished pulmonary expression of BMPR2 (Atkinson et al., 2002; Takahashi et al., 2006; Morty et al., 2007) and K_v1.5, K_v3.1 and K_v2.1 channels (Yuan et al., 1998; Bonnet et al., 2006; Guignabert et al., 2006). In diabetic animals lung

BMPR2 protein was downregulated as it has been also reported in diabetic rat kidneys (Wang et al., 2001). Our data are consistent with the concept that BMPR2 mutation or downregulation is a predisposing factor but may not be sufficient for PAH 5-HT (Long et al., 2006). In contrast, in diabetic rats there was no significant change in K_V currents and in $K_{V1.5}$ expression. This is consistent with the small, if any, change in both parameters found in the fawn hooded rats, an animal model of genetic predisposition to PAH, at 20 weeks of age (pre-hypertensive) (Bonnet et al., 2006).

Hyperresponsiveness to 5-HT in large and small PA rings is a common feature of animal models of PAH, including cardiopulmonary bypass-, chronic hypoxia-, intermittent hypoxia- or monocrotaline-induced PAH (Brown et al., 1998; Sato et al., 2000; Keegan et al., 2001; Thomas and Wanstall, 2003; Rodat et al., 2007). We also found a marked increase in the response to 5-HT in intrapulmonary arteries from diabetic rats. Because PA from $BMPR2^{+/-}$ mice also exhibit increased contractile responses to 5-HT (Long et al., 2006) it seems likely that, in the diabetic rats, hyperresponsiveness to 5-HT is a consequence of the BMPR2 downregulation. Interestingly, PAH is not evident in $BMPR2^{+/-}$ mice but it does develop after chronic 5-HT infusion, an effect that is exaggerated under hypoxic conditions (Long et al., 2006).

The increased response to 5-HT was maintained in endothelium-denuded vessels or in the presence of L-NAME, indicating that a major component of this phenomenon is not related to acute release of endothelial vasoactive factors. However, differences tended to be smaller in endothelium denuded compared to intact vessels suggesting that endothelial dysfunction (Al-Shafei et al., 2002; Lopez-Lopez et al., 2008) might also play a role. Moreover, it seems to be secondary to the high blood glucose rather than a

direct effect of streptozotocin since it was reduced by co-treatment with insulin. On the other hand, the similar potency of ketanserin, a specific 5HT_{2A} receptor competitive antagonist, on 5-HT contraction indicates that the responses to 5-HT are mainly mediated by 5HT_{2A} receptors in both control and diabetic animals. However, a possible additional role for other receptors such as 5-HT_{1B} cannot be ruled out (MacLean et al., 2000). The overexpression of 5HT_{2A} receptors in diabetic animals could be responsible for the higher contractile response of 5-HT. Nevertheless, this possibility does not explain the acute reversal by COX inhibitors or reactive oxygen species scavengers as discussed below.

COX-2 protein levels are increased in lungs from rats with PAH induced by hypoxia (Chida and Voelkel, 1996) or by high pulmonary blood flow (Sato et al., 2000; Lam et al., 2005) and in hypoxic human PA smooth muscle cells (Yang et al., 2002). In addition, elevated TXA₂ levels have been demonstrated in several forms of PAH (Christman et al., 1992). Accordingly, we found increased COX-2 expression in the PA from diabetic rats. Moreover, streptozotocin-induced diabetes led to an exaggerated lung production of PGE₂, PGF_{2 α} and PGD₂ from exogenous arachidonic acid (Watts et al., 1982). However, whether COX-2 is beneficial or detrimental in PAH is controversial. Thus, inhibition of COX-2 by celecoxib exhibited beneficial effects against the development of monocrotaline-induced PAH (Rakotoniaina et al., 2008). In contrast, hypoxia-induced PAH was exacerbated by the celecoxib derivative SC236 (Pidgeon et al., 2004) and in COX-2 knockout animals (Fredenburgh et al., 2008) (Cathcart et al., 2008). Herein, we found that acute inhibition of COX-2 can prevent the hypercontractile response to 5-HT in diabetic rats as previously found in PAH induced by intermittent hypoxia (Thomas and Wanstall, 2003) or by high pulmonary blood flow

(Sato et al., 2000). These results suggest that a COX-2 derived metabolite is responsible for the enhanced maximal response to 5-HT. The fact that the TP receptor agonist U46619 reproduces at least partially the effect of diabetes, suggests that the COX-2 metabolite might be acting on these receptors. Similarly, COX-2 dependent and endothelium-independent vascular hyperresponsiveness has also been reported in several systemic arteries from animal models of type 1 and type 2 diabetes (Jarajapu et al., 2008; Shi and Vanhoutte, 2008). Moreover, COX-2 inhibitors and ROS scavengers prevent the vascular hyperresponsiveness in endothelium denuded femoral arteries from streptozotocin-induced diabetic rats (Shi & Vanhoutte, 2008), suggesting that COX metabolites and ROS are generated in the smooth muscle. In addition, the TP receptor antagonist terutroban also prevented the femoral vascular hyperresponsiveness to ROS in diabetic animals, suggesting that the COX metabolite is acting via TP receptors (Shi & Vanhoutte, 2008).

Type 1 and type 2 diabetes are associated with systemic oxidative stress (Keaney and Loscalzo, 1999; Meigs et al., 2007). In PA, diabetes induces an increase in superoxide and upregulation of p47^{phox}, the regulatory subunit of the superoxide generating enzyme NADPH oxidase, which is involved in endothelial dysfunction (Lopez-Lopez et al., 2008). The downregulation of BMPR2 in diabetic rat kidney was prevented by the antioxidant tiron (Yeh et al., 2009), suggesting that it was secondary to oxidative stress. In addition, oxidative stress is also involved in the exaggerated response to vasoconstrictors in systemic arteries from diabetic animals (Shi and Vanhoutte, 2008). Thus, we hypothesized that scavenging superoxide using SOD or inhibiting its main source using apocynin, a widely used yet non selective NADPH oxidase inhibitor, might also prevent 5-HT hyperresponsiveness. In fact, both approaches prevented the

exaggerated response to 5-HT in diabetic PA without affecting the controls. On the contrary, exogenous addition of a superoxide donating drug such as pyrogallol increased the maximal response to 5-HT in control PA, mimicking the effects of diabetes.

The relationship of COX-2 and reactive oxygen species is complex because COX-2 can generate superoxide directly or indirectly via the release of TXA₂ (Cogolludo et al., 2006a; Shi and Vanhoutte, 2008) and conversely, COX-2 activity can be stimulated by reactive oxygen species (Garcia-Redondo et al., 2009). Thus, we questioned what was first in the signaling pathway in diabetic PA, COX-2 activity or increased oxidative stress. Our data fit better with the second possibility since indomethacin prevented the pyrogallol-induced hyperresponsiveness to 5-HT in control rats while apocynin had no effect on U46619-induced sensitization. Taken together, the present results suggest that NADPH oxidase-derived reactive oxygen species activate its downstream effector COX-2 leading to the enhanced response to 5-HT. This is further supported by the upregulation of the main proteins involved in this pathway, p47^{phox}, COX-2 and 5-HT_{2A} receptors observed in the diabetic lungs.

In conclusion, consistent with data in humans and animal models of PAH, in diabetic rats BMPR2 expression was downregulated, 5-HT_{2A} receptors, p47^{phox} and COX-2 were upregulated and PA were hyperresponsive to 5-HT. This latter effect was independent of the endothelium and appears to be related to NADPH oxidase-induced superoxide production and COX-2 derived metabolites. All these changes were not sufficient to induce a consistent increase in PA pressure or PA muscularization. However, a

prolonged period of diabetes induced an increase in RVSP. Our results further strengthen the link between diabetes and PAH.

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Authorship Contributions

JG L-L and J F-H performed the vascular reactivity, J M-S did the electrophysiological experiments and western blots, MJ G-V did the blood pressure measurements, GF and CM performed the PCRs and western blots, LM did the histology, LM, AC and JAL contributed to the design, analysis and discussion, F.P-V conceived the study and wrote the manuscript.

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Footnotes:

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Legends for Figures

Figure 1. Effects of diabetes on RVSP, right ventricular hypertrophy and PA muscularization. (A) Systolic and diastolic systemic arterial pressure (SAP), right ventricular systolic pressure (RVSP), right ventricular weight relative to body weight (RV/BW) and right ventricular weight relative to left ventricular plus septum weight [RV/(LV+S)] in control (open columns) and streptozotocin treated (solid columns) rats (n=5 and 8, respectively). (B) Percentage of muscular, partially muscular and non muscular arteries in control and diabetic rats (n=4). Each column represents the mean \pm SEM.

Figure 2. Diabetes downregulates BMPR2 but not Kv1.5 expression. Expression of (A) BMPR2 and (B) Kv1.5 in lungs from diabetic (d) and parallel control (c) rats measured by Western blot. (C) K_V currents recorded in isolated PA smooth muscle cells. Current traces (left) are shown for depolarization pulses from -60 mV to +60 mV from a holding potential of -60 mV. Current-voltage relationships (right) measured at the end of the pulse (n=4-8). * indicates $P < 0.05$ vs control.

Figure 3. PA from diabetic rats are hyperresponsive to 5-HT. Vasoconstrictor responses induced by 5-HT in endothelium-intact (+E) PA from diabetic and control rats in the absence (A, n=17 and n=13, respectively) or in the presence (B, n=7 and n=5, respectively) of L-NAME (10^{-4} M). (C) E_{max} and pD_2 values calculated from data on Fig 3A, 3B and in endothelium denuded (-E, from Fig 4A). ** indicates $P < 0.01$ vs control. # and ## indicate $P < 0.05$ and $P < 0.01$, respectively vs +E.

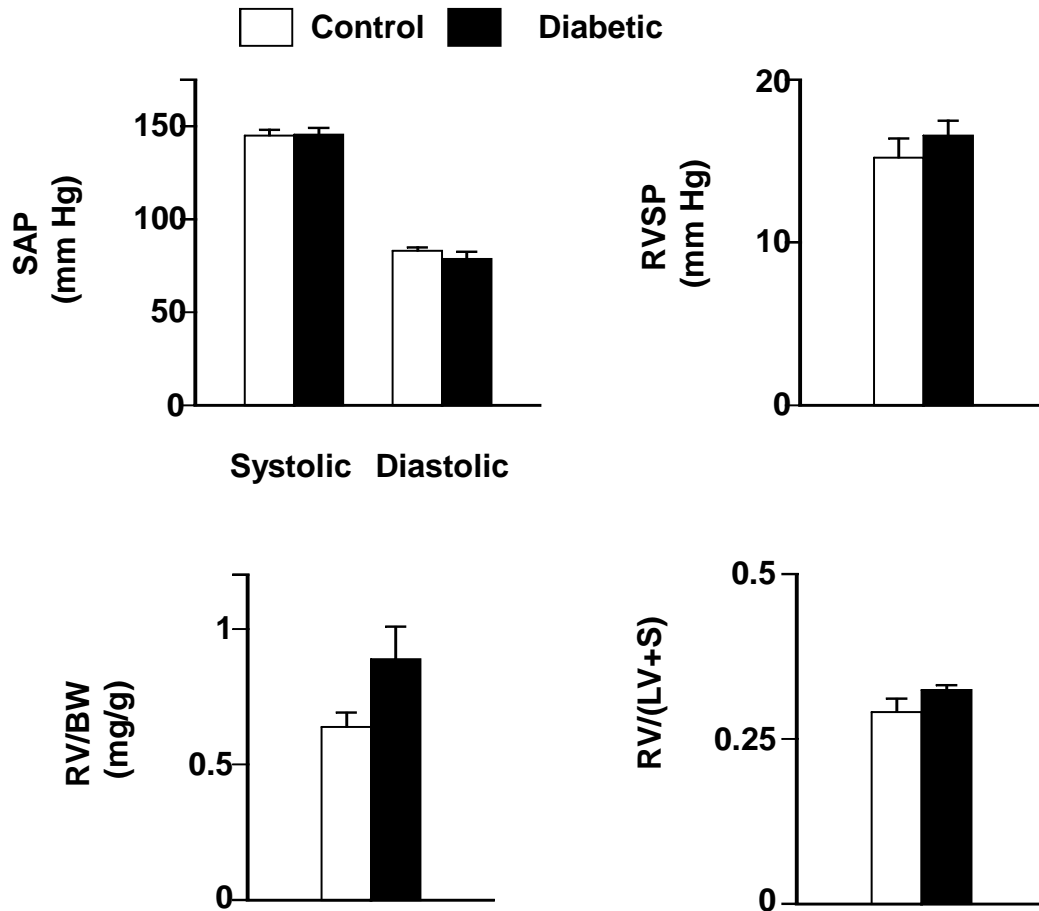
Figure 4. Role of 5-HT_{2A} receptors. (A) Effects of the 5-HT_{2A} receptor antagonist ketanserin (10^{-7} M) on the vasoconstrictor responses to 5-HT in endothelium denuded PA (n=5-7). (B) Calculated pD₂ and pK_B values for both endothelium-intact (+E) and endothelium denuded (-E) PA. (C) Expression of 5-HT_{2A} receptors in lungs from diabetic rats (d, n=8) and parallel controls (c, n=8). ** denotes P<0.01 vs control.

Figure 5. Role of COX-2. Effects of (A) the nonselective COX inhibitor indomethacin (INDO, 10^{-5} M) and (B) the COX-2 selective inhibitor NS-398 (10^{-5} M) on the vasoconstrictor responses to 5-HT in endothelium-intact PA (n=6). (C) E_{max} and pD₂ values calculated from panels A and B and in the absence of drug (untreated, calculated from Fig 3). (D) Expression of COX-2 in PA from control and diabetic rats (n = 5 and 6, respectively). *,** denote P<0.05 and P<0.01, respectively, diabetic vs control rats and † denotes P<0.05 indomethacin vs untreated.

Figure 6. Role of superoxide. Effects of (A) the superoxide scavenger superoxide dismutase (SOD, 100 U/ml) and (B) the NADPH oxidase inhibitor apocynin (3×10^{-4} M) on the vasoconstrictor responses to 5-HT in endothelium intact PA (n=6).

Figure 7. Superoxide and a TXA₂ analog enhance 5-HT contractile responses in PA from control rats. Effects of (A) the non enzymatic generator of superoxide pyrogallol (pyrog, 3×10^{-6} M) in the absence or presence of indomethacin (indo, 10^{-5} M) and (B) the TXA₂ analog U46619 (10^{-8} M) in the absence or presence of apocynin (apoc, 3×10^{-4} M) on the vasoconstrictor responses to 5-HT in PA (n=4-8). ** denotes P < 0.01 vs control.

A



B

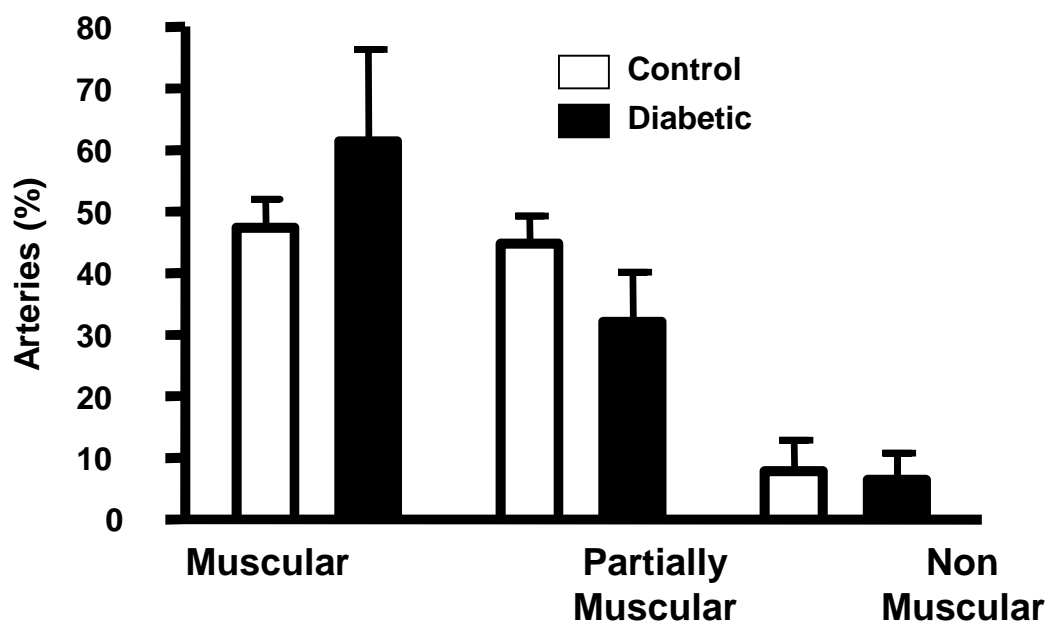


Fig. 1.

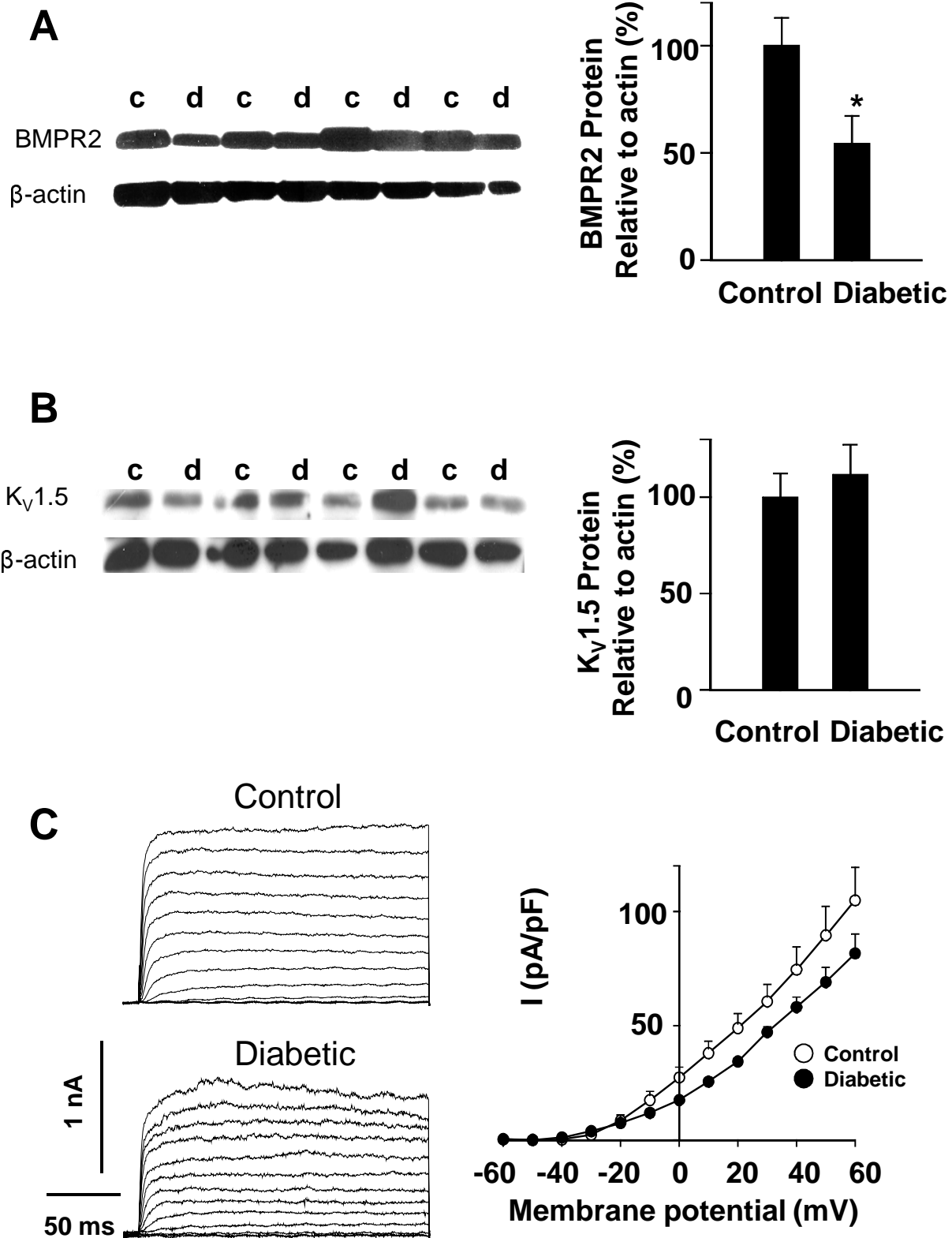


Fig. 2.

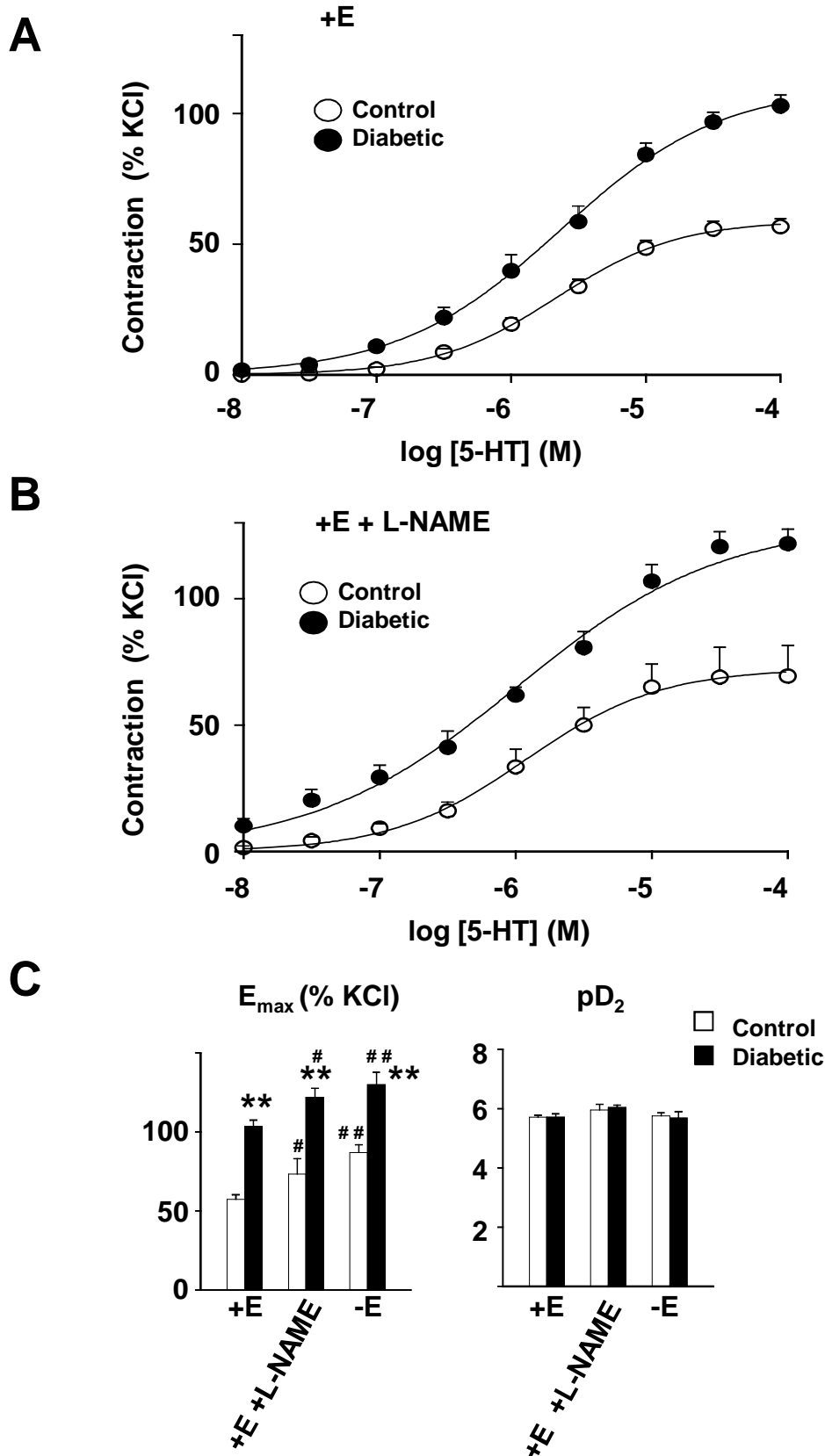
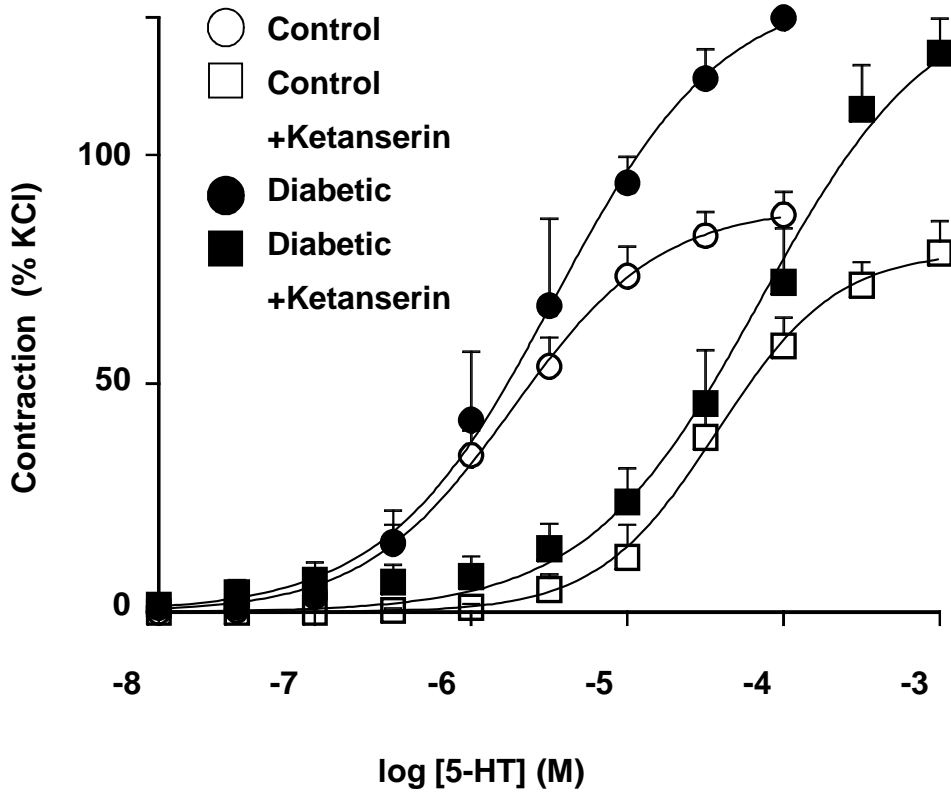
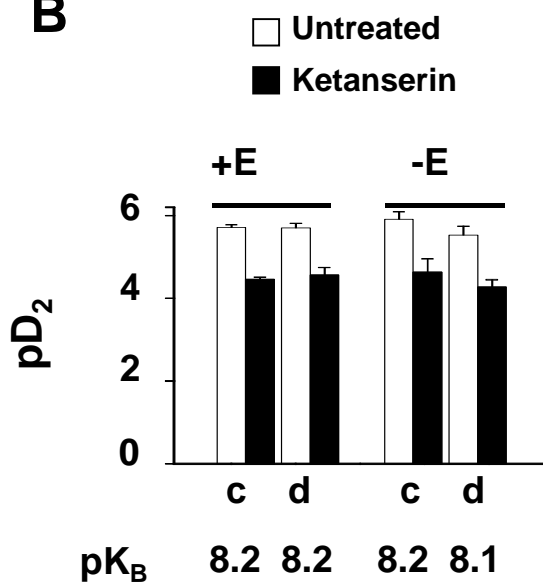


Fig. 3.

A



B



C

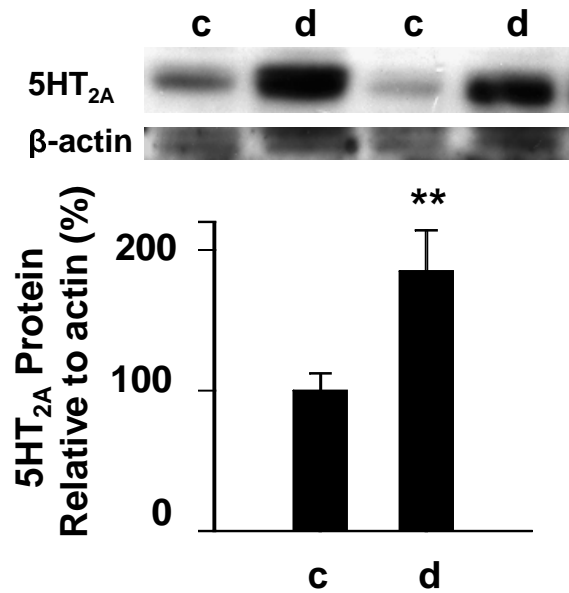


Fig. 4.

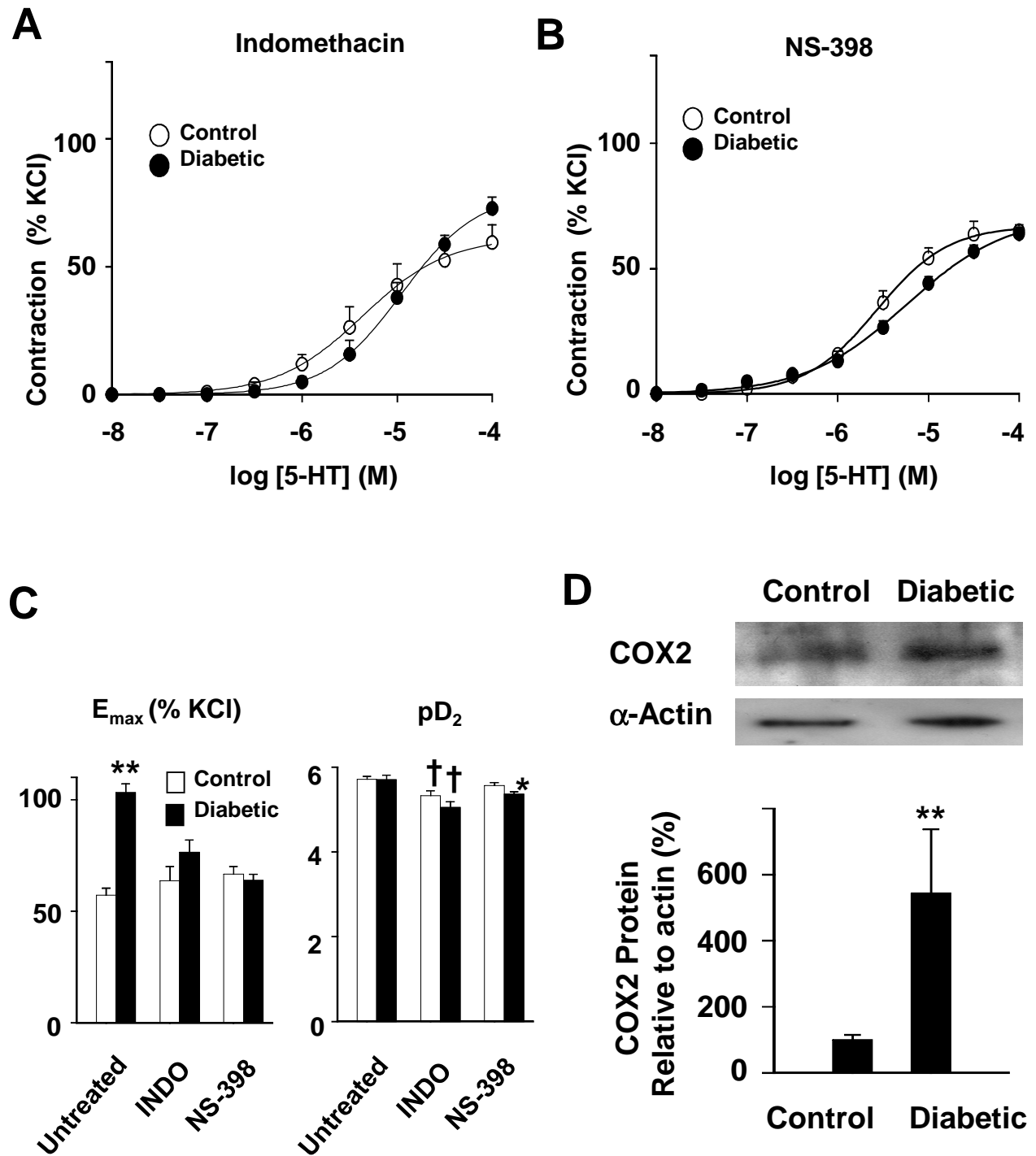
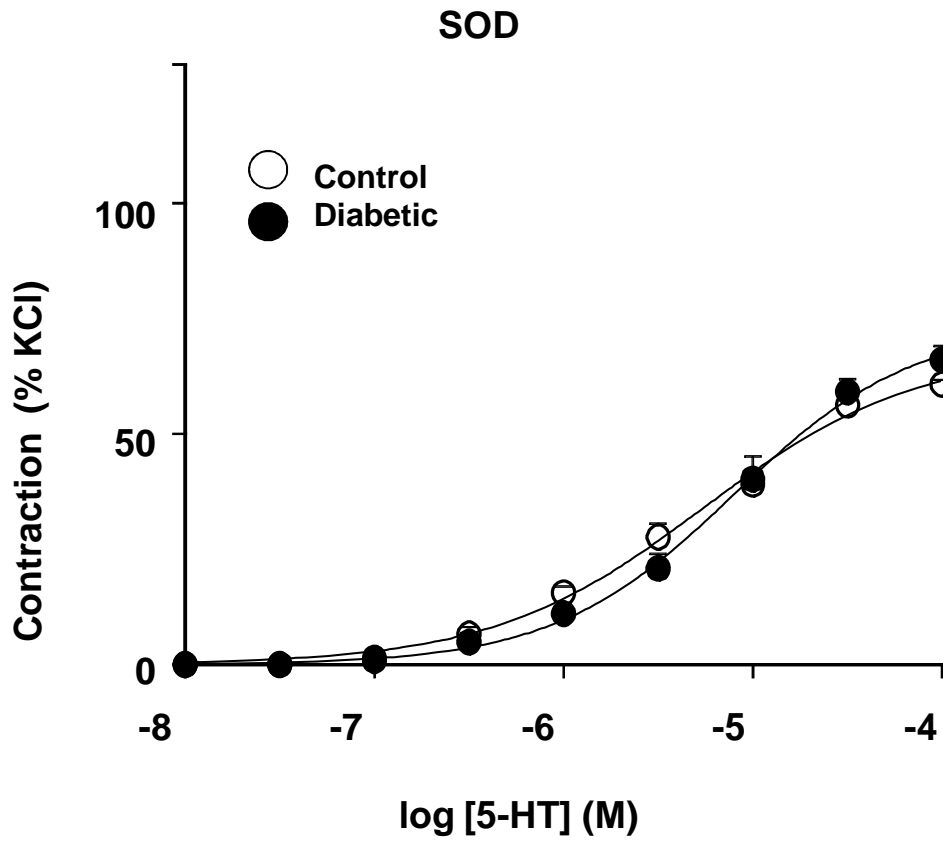


Fig. 5.

A



B

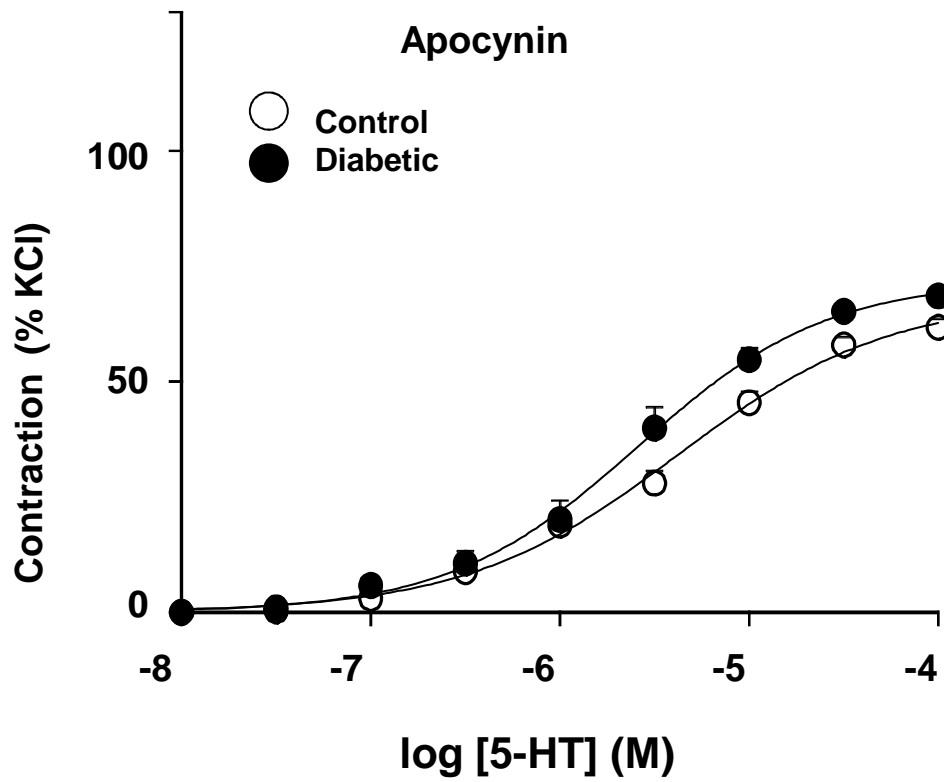
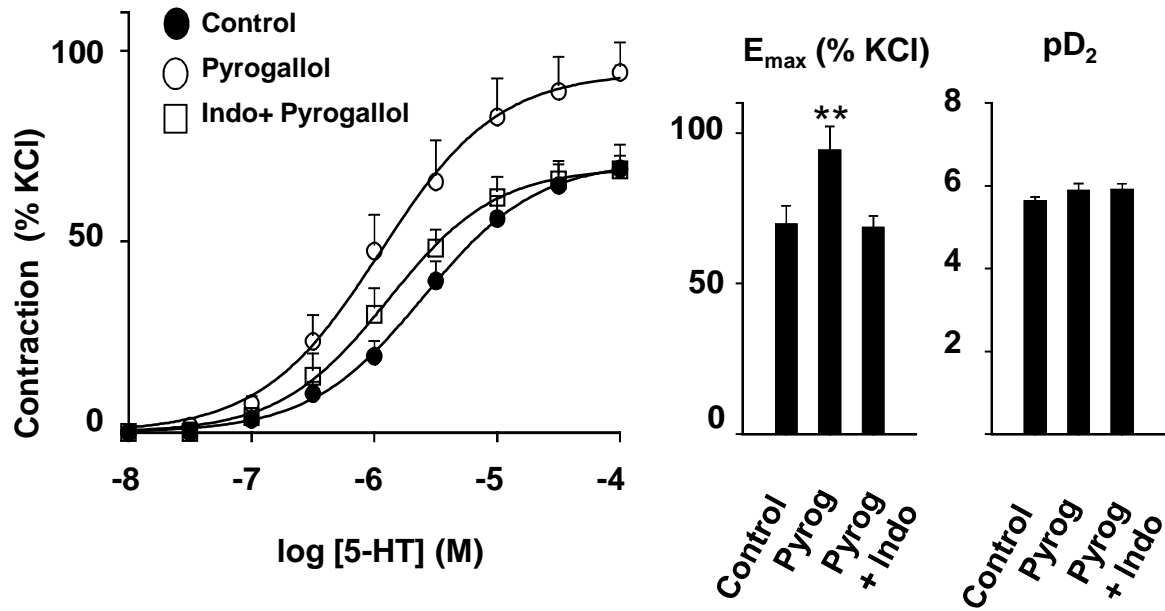


Fig. 6.

A



B

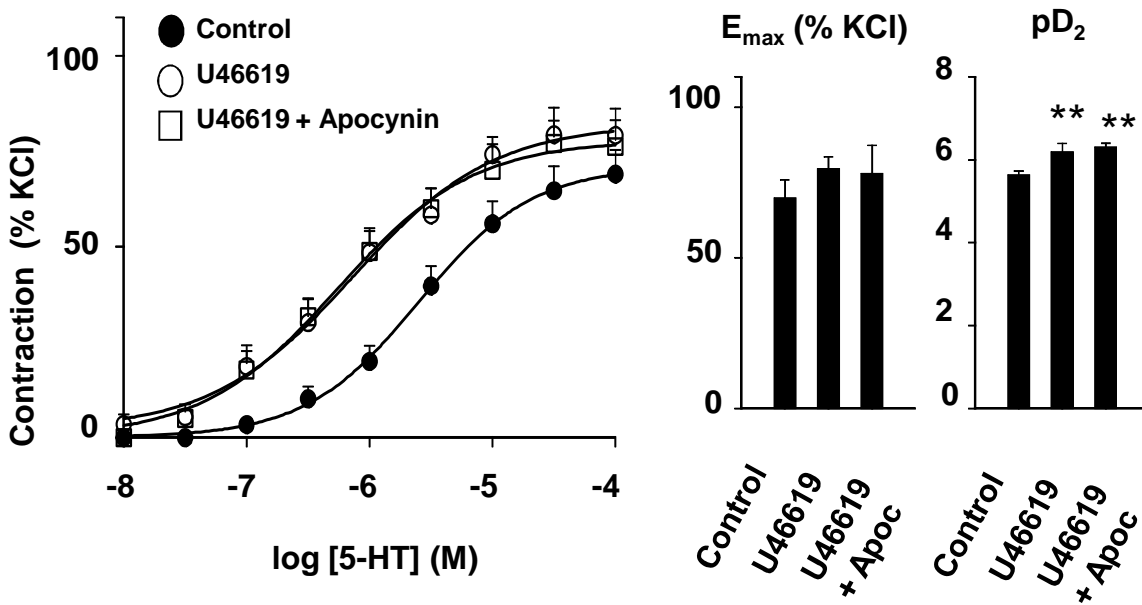


Fig. 7.