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Metformin treatment improves erectile function in an angiotensin II model of erectile dysfunction

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Abstract

Introduction—Increased angiotensin II (AngII) levels cause hypertension, which is a major risk factor for erectile dysfunction (ED). Studies have demonstrated that increased AngII levels in penile tissue are associated with ED. A recent study showed that metformin treatment restored nitric oxide synthase (NOS) protein expression in penile tissue in obese rats; however, whether metformin treatment can be beneficial and restore erectile function in a model of ED has not yet been established.

Aim—The goal of this study was to test the hypothesis that AngII induces ED by means of increased corpus cavernosum contraction, and that metformin treatment will reverse ED in AngII-treated rats.

Methods—Male Sprague-Dawley rats were implanted with mini-osmotic pumps containing saline or AngII (70 ng/min, 28 days). Animals were then treated with metformin or vehicle during the last week of AngII infusion.

Main Outcome Measures—Intracavernosal pressure (ICP); corpus cavernosum contraction and relaxation; nNOS protein expression; extracellular signal-regulated kinase (ERK1/2), AMP-activated protein kinase (AMPK) and eNOS protein expression and phosphorylation.

Results—AngII induced ED was accompanied with an increase in corpus cavernosum contractility, decreased nitroergic relaxation and increased ERK1/2 phosphorylation. Metformin treatment improved erectile function in the AngII-treated rats by reversing the increased contraction and decreased relaxation. Metformin treatment also resulted in an increase in eNOS phosphorylation at ser1177.

Conclusions—Metformin treatment increased eNOS phosphorylation and improved erectile function in AngII hypertensive rats by re-establishing normal cavernosal smooth muscle tone.

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Keywords

Angiotensin II; Erectile Dysfunction; Corpus Cavernosum; Hypertension; Metformin; eNOS

Introduction

Hypertension is considered a risk factor for a variety of other cardiovascular conditions, and has been demonstrated to contribute to the development of erectile dysfunction (ED). In fact, some studies demonstrated that while the occurrence of ED in the general population is estimated at 9.6%, 30% of hypertensive patients have ED^{1, 2}. ED is recognized by the World Health Organization as a quality of life disorder that needs to be treated³. ED is now considered a disease which goes beyond life satisfaction and frustration; it is a warning sign for cardiovascular disease (CVD) and an indicator of overall endothelial dysfunction. Recent studies suggest that ED may be a clinical biomarker or a “warning sign” for underlying CVD, given that these two conditions frequently co-exist, with symptoms of ED presenting before the discovery of CVD^{4–7}. Other evidence suggests that ED increases in prevalence with aging and severity of CVD⁸. A common denominator in both hypertension and ED is the presence of endothelial dysfunction, which is characterized by decreased nitric oxide (NO) production and/or bioavailability and increased superoxide (O_2^-) production. In addition, there is a marked decrease in endothelium-dependent vasodilation or increases in endothelium-dependent contraction^{6, 9–11}.

A central player in hypertension, endothelial and vascular dysfunction is angiotensin II (AngII), which is the main effector of the renin-angiotensin-aldosterone system (RAAS). AngII is vital to the regulation of salt and water homeostasis, and therefore a main contributor to blood pressure. However, increased AngII levels have been shown to induce excessive vasoconstriction, endothelial dysfunction, vascular remodeling, and promote insulin resistance. Noticeably, all these characterize a variety of diseases such as atherosclerosis, hypertension and diabetes. In addition, with new evidence demonstrating a role for the RAAS system in penile tissue, this suggests that AngII itself may modulate penile function; with excessive production and dysfunctional signaling leading to ED^{11–14}.

Traditionally, AngII was considered to work in an endocrine manner; however, paracrine actions of AngII have been discovered, with local tissues and organs producing AngII in concert with its cognate receptors, AT_1 and AT_2 ¹⁵. Activation of the AT_1 receptor leads to the stimulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. This enzyme is central in the production of reactive oxygen species (ROS). ROS is known to be detrimental to the endothelium and smooth muscle due to the direct scavenging of available NO necessary for vasorelaxation, as well as contributing to vascular damage¹⁶. Furthermore, ROS can also stimulate the RhoA/Rho-kinase pathway leading to paralleled increases in vasoconstriction^{17–20}.

Metformin is an oral biguanide classically used for the treatment of diabetes²¹. In addition to its anti-hyperglycemic role, metformin was shown to lower blood pressure in hypertensive patients and animals as well as improving vascular and endothelial function. Furthermore, metformin has been suggested to possess anti-inflammatory and anti-oxidative

properties^{22–26}. Recently, a paper by Kim *et al.* has shown that metformin treatment restored the penile expression of eNOS and nNOS in rats fed a high fat diet²⁷. This makes metformin a candidate for use in the treatment of AngII-mediated ED. In this study, we hypothesize that chronic AngII treatment leads to ED through increased cavernosal contractility, and that treatment with metformin will restore erectile function in an AngII model of ED.

Methods

Animals

Male Sprague-Dawley (SD) rats (12–14 weeks old, Harlan Laboratories, Indianapolis, IN, USA) were used in the present studies. All procedures were performed in accordance with the Guiding Principles in the Care and Use of Animals and approved by the Georgia Health Sciences University Committee on the Use of Animals in Research and Education. The animals were housed four per cage on a 12-h light/dark cycle and fed a standard chow diet with water *ad libitum*.

Surgical procedures and osmotic mini-pump (Alzet, Durect Corp Cupertino, CA, USA) implantation were performed using isoflurane anesthesia (4% isoflurane in 10% oxygen [O₂]) and under sterile conditions. Rats were infused with AngII (70 ng/minute) for 4 weeks or sham surgery performed¹⁸. Each group of rats, AngII-treated and sham, were divided into 2 sub-groups: metformin-treated and vehicle-treated. Metformin (500 mg/kg/d) was dissolved in the drinking water and administered orally (via gavage) for the last 7 days of AngII infusion^{18, 22}.

Blood Glucose Measurement

Rats were fasted for 5–6 hours, and blood was drawn from tail vein. Glucose was measured by a commercially available glucose meter (Accu-Check Active, Indianapolis, IN, USA)²⁸.

In vivo measurement of Intracavernosal Pressure/Mean Arterial Pressure

Changes in intracavernosal pressure (ICP) in response to electrical stimulation of the cavernosal nerve were assessed in sham and treated animals, as previously described^{28–30}. Animals were anaesthetized with 4% isoflurane in 10% O₂. To monitor and calculate mean arterial pressure (MAP) and ICP, the left femoral artery and the right crura were cannulated with polyethylene (PE)-10 tubing tied with 6–0 silk suture. This was fitted to a heparinized saline-filled pressure transducer. The major pelvic ganglion was accessed via a midline incision and stimulated with bipolar silver electrodes. ICP changes were monitored in response to 0.2–20 Hz (5 ms pulses at 6 V). The stimulations for each frequency lasted 45 s. ICP and arterial pressure were converted from analog to digital signals and transmitted to a data-acquisition program (Chart software, version 5.2; ADI Instruments, Colorado Springs, CO). The erectile response was calculated using maximum ICP response at each stimulation frequency normalized to MAP at the time of ICP measurement^{28, 29}.

Functional Studies in Cavernosal Strips

After carbon dioxide (CO₂) euthanasia, penes were excised, transferred into ice-cold physiological salt solution ([PSS], 130 mM NaCl, 14.9 mM NaHCO₃, 5.5 mM dextrose, 4.7 mM KCl, 1.18 mM KH₂PO₄, 1.17 mM MgSO₄·7H₂O, 1.6 mM CaCl₂·2H₂O, and 0.026 mM ethylenediaminetetraacetic acid [EDTA]), and dissected to remove the tunica albuginea, as previously described^{28, 29}. A single strip preparation was obtained from each corpus cavernosum. Cavernosal strips were mounted in 4-mL muscle myograph chambers (Danish Myo Technology, Aarhus, Denmark) containing buffer at 37°C continuously bubbled with a mixture of 95% O₂ and 5% CO₂. The tissues were stretched to a resting force of 3.0 millinewtons (mN) and allowed to equilibrate for 60 minutes. Changes in isometric force were recorded using a PowerLab/8SP data acquisition system (Chart software, version 5.0; ADInstruments, Colorado Springs, CO, USA). After stabilization, tissues were contracted with high potassium chloride (KCl) solution (120 mM) to verify the contractile ability of the preparations.

Concentration-response curves (CRC) to PE (10nM to 100µM) were performed to determine cavernosal contractility. EFS stimulation of cavernosal strips was used to assess nerve-mediated contractility. EFS is applied to strips placed between platinum pin electrodes attached to a stimulus splitter unit (Stimu-Splitter II) and connected to a Grass S88 stimulator (Astro-Med West Warwick, RI). EFS was conducted at 20 V, 1-ms pulse width and trains of stimuli lasting 10 s at varying frequencies (1 to 64 Hz). To evaluate adrenergic nerve-mediated responses, strips were incubated with L-NAME (10⁻⁴ M) plus atropine (10⁻⁶ M) before EFS was performed^{28, 29}.

Drugs and Solutions

Atropine, N^G-nitro-L-arginine methyl ester (L-NAME) and PE were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Stock solutions were prepared in deionized water, and stored in aliquots at -20°C; dilutions were prepared immediately before use. Metformin was purchased from MP Biomedicals.

Statistical Analysis

For PE, concentration-response curves (CRC) were fitted using a nonlinear interactive fitting program (GraphPad Prism, Graph Pad Software Inc., San Diego CA), and values expressed as a difference between basal tone and force generation (in mN). Maximum responses are expressed as (*E*_{max}). Curves performed to EFS were plotted as force generation (in mN) for each voltage point. Data are expressed as means±SEM (n), where n is the number of experiments performed. Statistical analysis of agonist CRCs was performed using the *F*-test for *E*_{max} values obtained from best-fit analysis. Statistical analysis of EFS curves was performed by using repeated measures one-way analysis of variance (ANOVA), with post-hoc Bonferoni's test, for comparison between the groups. ICP/MAP values were assessed for statistical significance using two-way ANOVA, with post-hoc Bonferoni's test. Blood glucose, body weight and blood pressure were analyzed using one-way ANOVA test. Values of *p*<0.05 were considered a statistically significant difference.

Results

Effect of AngII and metformin on metabolic parameters and mean arterial pressure

AngII infusion significantly increased blood pressure compared to sham-treated rats. Treatment with metformin had no effect on MAP in sham rats. Although AngII rats treated with metformin remain hypertensive, metformin treatment significantly attenuated MAP in AngII-treated rats, but did not normalize it back to normal levels (Figure 1A).

AngII infusion caused a mild hyperglycemia in rats treated with AngII for 4 weeks when compared to sham rats. Treatment with metformin normalized blood glucose levels in AngII-treated rats, without significantly affecting blood glucose levels in sham rats (Figure 1B).

AngII infused rats exhibited a significant decrease in body weight compared with sham animals, which has been shown in previous studies³¹. Also, treatment with metformin caused a significant decrease in body weight in sham animals. Previous studies have also shown that metformin treatment was associated with decreased body weight^{22, 32}. However, metformin did not lead to significant change in body weight in AngII-treated rats (Figure 1C).

Metformin treatment improves erectile function in AngII-infused rats

Erectile function was assessed by measuring ICP upon stimulation of the pelvic ganglion, which was then normalized to MAP^{28, 29}. To determine the effects of metformin treatment alone, we assessed the erectile function in sham rats treated with metformin; we did not observe any differences when compared to sham rats treated with metformin. These data suggest that metformin treatment does not affect erectile function in healthy rats (Figure 2).

AngII infusion impaired erectile function when compared to animals receiving sham surgery, which is in accordance with a previous study published in 2008 by Jin and colleagues¹⁸. This impairment was characterized by a decrease in the maximum ICP/MAP response in AngII-treated rats when compared to sham animals. Metformin treatment significantly increased MaxICP leading to improvement of the erectile response in AngII-treated rats (Figure 2). Metformin treatment alone did not affect either MAP or erectile function in sham rats. We next wanted to explore the mechanism through which AngII causes ED and how metformin may be improving erection in AngII rats

Metformin treatment reversed the Ang II increased phenylephrine (PE)- and electric field stimulation (EFS)-mediated contraction in the corpus cavernosum

To determine the mechanism of the AngII-mediated decrease in erectile function, cavernosal reactivity was assessed in AngII-treated and sham-treated rats. We first determined adrenergic receptor activation in cavernosal smooth muscle cells by performing a CRC to PE. In cavernosum from AngII infused animals, we observed a significant increase in maximum contraction (E_{max}) compared to corpus cavernosum from sham rats (E_{max} 5.492 ± 0.332 mN in AngII vs E_{max} 3.764 ± 0.141 in sham (Figure 3A). Considering that the penile erectile response is under neuronal control, it was important to assess the contractile

response induced by EFS. Cavernosal strips were incubated with the NOS inhibitor, L-NAME (0.1mM), and the muscarinic antagonist, atropine (1μM) to evaluate adrenergic nerve-mediated responses, we observed that cavernosal strips isolated from AngII-treated rats exhibited a significantly increased contractility when compared to corpora cavernosa from sham surgery rats (Figure 3B). These data suggest that a mechanism by which AngII may cause ED is via increased cavernosal smooth muscle contraction.

Metformin treatment reversed the increased contraction of corpus cavernosum in response to PE (Figure 3A) and EFS (Figure 3B) back to normal levels, reversing the responses to comparable sham levels. These data suggest that metformin treatment can lead to a reversal of AngII-induced ED, as well as decrease the contractility of the corpus cavernosum smooth muscle in penile tissue.

Metformin treatment improved NANC relaxation of corpus cavernosum from AngII-treated rats

NANC stimulation resulted in a frequency dependent relaxation in rat corpus cavernosum. The nitrenergic mediated relaxation in corpus cavernosum isolated from AngII-infused rats in response to electric field stimulation was decreased when compared to sham animals ($p<0.05$). However, when compared to corpus cavernosum isolated from AngII-infused rats, treatment with metformin improved and restored the NANC mediated relaxation in corpus cavernosum from AngII-infused rats treated with metformin (Figure 4).

ERK1/2 phosphorylation is increased in the corpus cavernosum of AngII-treated rats

Corpus cavernosum isolated from AngII-infused rats exhibited a significant increase in phosphorylation of ERK1/2 when compared with sham rats. ERK1/2 phosphorylation in AngII rats tended to decrease with metformin treatment, although these results were not statistically significant from the sham and AngII-infused groups (Figure 5).

Increased AMPK phosphorylation correlates with an increase in eNOS phosphorylation with metformin treatment with no effect on nNOS expression

Interestingly, AngII infusion alone significantly increased phosphorylation of AMPK, which has been shown to be upstream eNOS. Co-treatment with metformin also increased AMPK phosphorylation at threonine 172 residue, however, there was no significant difference between AngII only and AngII + metformin treated groups (Figure 6). AngII treatment did not affect eNOS phosphorylation at the serine 1177 residue in the corpus cavernosum; however, co-treatment with metformin significantly increased eNOS phosphorylation at ser 1177 (Figure 7). Neither AngII alone nor co-treatment with AngII and metformin affected the nNOS expression in the rat corpus cavernosum. These data suggest that metformin treatment may be leading to the activation of eNOS in the cavernosum, possibly increasing NO bioavailability, thus decreasing contractility and increasing relaxation.

Discussion

In the present study, we investigated the effect of metformin on a model of AngII-induced ED. We found that metformin treatment improved erectile function in an AngII hypertensive

model of ED and reversed AngII-mediated increases in corpus cavernosum contractility. AngII infusion caused a significant increase in MAP and treatment with metformin attenuated the AngII-induced hypertension in rats, whereas it did not affect MAP in the sham rats.

These results corroborate other studies showing that metformin does not affect arterial pressure in normal subjects^{24, 33}. However, the anti-hypertensive effects of metformin have been reported in hypertensive patients as well as various hypertensive animal models^{23, 24, 34, 35}. In this study, we found that not only AngII treatment, but animals given metformin had a decreased body weight as compared to sham rats. This has been well documented by other investigators.^{22, 32, 31, 36} However, there was no further reduction in body weight in those animals co-treated with metformin. We also observed that AngII infusion caused a mild hyperglycemia. These data were not surprising as clinical and pharmacological studies have demonstrated that AngII is a critical promoter of insulin resistance and can induce type II diabetes mellitus. Coincidentally, both are also risk factors for ED^{37–40}. We found that metformin treatment was able to restore blood glucose to normal levels in AngII-treated rats, in accordance with its anti-hyperglycemic and insulin-sensitizing action^{34, 41–43}. As expected, metformin did not affect blood glucose level in sham animals. Although not investigated in this manuscript, there are well-investigated mechanisms for AngII-induced hyperglycemia via actions on insulin-sensitive tissues such as liver, muscle and adipose tissue, modulating the activity of various signaling molecules downstream of the insulin signaling pathway^{37, 39}. Since metformin treatment did not affect metabolic parameters or MAP, as well as having no discernable effects on erectile function, in sham rats we further investigated only the effects of metformin treatment in AngII-hypertensive rats.

Previous studies have shown that excessive production of AngII is associated with ED^{11, 13, 17, 18}. Studies have also shown that AngII levels are increased in the cavernous blood of patients with ED, as compared with healthy subjects¹³. Jin *et al.* demonstrated that AngII infusion (70ng/min for 4 weeks) caused ED in rats, and they proposed a mechanism through which AngII may lead to ED.^{18, 44} This mechanism postulates that increased AngII leads to an increase in NADPH oxidase activation and NADPH oxidase-dependent ROS generation via the AT₁ receptor. Increased ROS generation, in turn, activates RhoA/Rho-kinase signaling, thus inhibiting eNOS. This process results in increased cavernosal smooth muscle contraction, contributing to the pathogenesis of ED⁴⁴. Others have found active phospho-ERK1/2 to be elevated in the corpus cavernosum of patients with ED when compared to those patients with normal erectile function⁴⁵.

Metformin is an anti-hyperglycemic drug which also exerts an anti-hypertensive effect, improves vascular and endothelial function in diabetic and hypertensive rats, and prevents oxidative stress as well as exhibiting anti-inflammatory and anti-atherogenic properties^{21, 22–26, 34, 46}. As mentioned previously, recent literature revealed that metformin treatment restored penile nNOS and eNOS expression in a model of high fat-fed obese rats³². These new findings led us to our hypothesis that treatment with metformin would restore erectile function in an AngII hypertensive model of ED. Indeed, we have observed that metformin treatment restored erectile function, as shown via ICP/MAP levels, in AngII-

treated rats. Moreover, the increased cavernosal contractility observed in the corpora cavernosa of AngII-treated rats was reduced to sham levels after metformin treatment, further supporting our hypothesis. In addition, this study revealed that metformin treatment also improved NANC relaxation. Our data support the theory that metformin treatment improves relaxation and reduces contractility in an AngII-treated rats, as well as improving erectile function.

To explore molecular mechanisms through which metformin may exert its beneficial effect, western blot analysis was performed on corpus cavernosum from AngII. Here we reveal that we observed increased phospho-ERK1/2, also downstream of AT₁ receptor activation. These data corroborate previous evidence from our laboratory demonstrating increased ERK1/2 in CC from DOCA-salt hypertensive mice, as well as CC from diabetic mice^{47, 48}. The increased ERK1/2 phosphorylation in corpus cavernosum from AngII-treated rats may provide an explanation to the observed increase in corpus cavernosum contractility of the AngII-infused rats.

It is widely accepted that a mechanism of action of metformin is through the AMP-activated protein kinase (AMPK) activation^{49, 50}. AMPK is a serine/threonine protein kinase, which is involved in the regulation of cellular, and organism metabolism. Of late, researchers have demonstrated the importance of AMPK in cardiovascular functions^{49, 51}. Our data showed that AngII infusion surprisingly led to an increase in phosphorylated AMPK and although not significant, we observed some further augmentation of phospho-AMPK levels with metformin treatment. These data were surprising, but others have found similar results. A recent paper by Nagata *et al.* has shown that AngII treatment induced AMPK activation in vascular smooth muscle cells (SMC); their data suggested that AMPK serves as a negative feedback system in AngII signaling by inhibiting the MAPK/ERK signaling downstream of AT₁R⁵². We believe that the increased AMPK levels in AngII-treated rats may be a compensatory system to counteract the negative effects of AngII in SMC. This suggests a beneficial effect of AMPK on vascular function.

Kim and colleagues showed that metformin treatment restored the penile expression of NOS, which is associated with AMPK activation³². In fact, activation of AMPK was shown to increase eNOS phosphorylation at serine (ser1177) and (ser633) leading to the activation of eNOS, thus increasing NO production^{53–55}. Furthermore, a recent study by Bae and colleagues demonstrated that phospho-eNOS is also increased using a novel AMPK activator, beta-lapachone, in a HUVEC cell culture model in addition to having a pronounced relaxatory effect in rabbit cavernosum.⁵⁶ In this study we did not see an effect of AngII nor metformin on the expression of nNOS; however, treatment with metformin did increase the phosphorylation levels of eNOS at ser1177. Taken together, our data suggest that AngII may be activating the ERK1/2 pathway, leading to increased CC contractility and that treatment with metformin may be increasing eNOS activity via AMPK.

Even though outside of the scope of this manuscript, there are other postulated mechanisms through which metformin may exert beneficial effects on erectile tissue; for example, through the reduction of intracellular production of ROS.^{46, 57} AngII-treated rats given apocynin, an NADPH oxidase inhibitor, have reduced ROS generation and improve erectile

function, suggesting that NADPH oxidase ROS production is a link in AngII-induced ED¹⁸. ROS is known to reduce NO bioavailability, and recent data from Ohmasa and colleagues showed that reduction in ROS, via edaravone, can improve NO signaling in the CC in diabetic rats⁵⁸. Similar to previously published work, we demonstrated that metformin treatment increases phospho-eNOS, possibly leading to NO generation. Increased NO may be beneficial in a model where there are increased ROS. Studies investigating the association between decreased NO production and ED have been performed in human populations; there is significant evidence demonstrating that a polymorphism in eNOS (G894T) results in a disturbance of eNOS activity and NO production. They also demonstrated that this polymorphism is associated with increased susceptibility to ED and metabolic syndrome⁵⁹. Increased NO can also prevent Rho-kinase activation via inhibition of the RhoA migration to the plasma membrane, thus decreasing corpus cavernosum contractility^{60, 61}.

There are a plethora of other possible mechanisms by which metformin treatment, via AMPK activation, could be leading to an improvement in our model. AMPK activation inhibits fatty acid-induced increases in nuclear factor κ B (NF- κ B) trans-activation and cytokine-induced NF- κ B activation in vascular endothelial cells, suggesting that it has beneficial effects on endothelial inflammation induced by deleterious stimuli⁶². A study by DiBona and colleagues found that metformin infusion has a sympathoinhibitory action, and they concluded that metformin inhibits peripheral sympathetic nerve activity by a central nervous system site of action.⁶³ In contrast AngII is sympathoexcitatory. Thus, it appears that all these mechanisms may work together to prevent AngII-induced increases in corpus cavernosum contractility, thus improving erectile function.

We believe that metformin treatment, possibly through activation of AMPK may represent an alternative therapeutic approach for ED associated with insulin resistance, diabetes, hypertension or other pathological conditions where increased endothelial or vascular dysfunction is present. Future studies which seek to determine the effectors through which metformin might exert its anti-ED action are necessary and will help to elucidate the important players governing erectile function, under both normal and pathological conditions. This will further our knowledge in the field of sexual medicine and may lead to the development of safe and novel therapeutic approaches to treat sexual dysfunction.

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Figure 1.

Changes in metabolic parameters and mean arterial pressure (MAP): (A) AngII-infused rats were hypertensive, which was attenuated with metformin treatment; however, no changes in MAP were observed in metformin treated sham rats. (B) AngII treatment resulted in mild hyperglycemia with blood glucose levels significantly higher than sham surgery. Normal blood glucose levels were restored in AngII-hypertensive rats given metformin. (C) AngII-treated rats exhibited a significant decrease in body weight when compared to sham. Treatment with metformin led to a significant reduction in body weight of sham rats; however, it did not affect body weight in AngII-treated rats. Results are mean $\pm$ SEM (n=4–14). * $p < 0.05$ vs. sham group; # $p < 0.05$ vs. AngII group.

Figure 2.

AngII treatment resulted in a decline of erectile function. *In vivo* assessment of maximal ICP/MAP responses to major pelvic ganglion stimulation was performed on sham and treated rats. Metformin treatment improved erectile function in AngII-infused animals, with no observed effects in sham rats. Data are represented as maximal ICP/MAP. Values represent mean $\pm$ SEM (n= 4–8). * p<0.05 vs. sham group; # p<0.05 vs. AngII group.

Figure 3.

Metformin treatment reduced the increased contractility seen in corpus cavernosum from AngII-treated rats in response to (A) concentration response curves (CRC to phenylephrine (PE) and (B) contraction to electric field stimulation (EFS). Contractility to PE and EFS was increased in corpus cavernosum of AngII-hypertensive rats. This response was restored back to sham levels in rats treated with metformin. Values represent mean $\pm$ SEM (n= 7–8 per group). * $p < 0.05$ vs. sham group; # $p < 0.05$ vs. AngII group.

Figure 4.

Metformin improves nitrgic relaxation in AngII-treated corpus cavernosum. EFS-mediated nitrgic relaxation was impaired in corpus cavernosum from AngII-hypertensive rats.

Animals co-treated with AngII and metformin exhibited an improved EFS-mediated nitrgic relaxation response in cavernosum. Values represent mean $\pm$ SEM (n= 4–8 per group). * p<0.05 vs. sham group; # p<0.05 vs. AngII group.

Figure 5.

AngII increases corporal ERK1/2 activity. Western blot analysis of phosphorylated (p)-ERK1/2 and total p38 MAPK expression in cavernosal tissue. AngII significantly increases corporal ERK1/2 phosphorylation, whereas metformin treatment tended to decrease ERK1/2 phosphorylation. Representative blot of p-ERK1/2, total ERK1/2; β-actin is shown in the top panel. Data are expressed as fold of sham expression (n=5–6). * $p < 0.05$ vs. sham group.

Figure 6. AMPK phosphorylation was increased with both AngII and metformin treatment. Western blot analysis revealed an increase in phosphor-AMPK in both AngII-hypertensive and hypertensive animals treated with metformin. Representative blot of p-AMPK, total AMPK; β-actin is shown in the top panel. Data are expressed as fold of sham expression (n=7–8). * p<0.05 vs. sham group.

Figure 7.

Metformin treatment increased the phosphorylation of eNOS at ser1177, but did not affect nNOS expression: Western blot analysis showed that AngII-hypertensive rats treated with metformin had a higher expression of phosphor-eNOS, as compared to AngII alone. Both treatments did not affect the expression of nNOS. Representative blots are shown in the top panel. Data are expressed as fold of sham expression (n=6–8). * p<0.05 vs. sham group; # p<0.05 vs. AngII group.