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Glucocorticoid-Induced Fetal Programming Alters the Functional Complement of Angiotensin Receptors Subtypes within the Kidney

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Abstract

We examined the impact of fetal programming on the functional responses of renal angiotensin receptors. Fetal sheep were exposed *in utero* to betamethasone (BMX; 0.17 mg/kg) or control (CON) at 80–81 days gestation with full term delivery. Renal nuclear and plasma membrane fractions were isolated from 1.0–1.5 year old sheep for receptor binding and fluorescence detection of reactive oxygen species (ROS) or nitric oxide (NO). Mean arterial blood pressure and blood pressure variability were significantly higher in the BMX-exposed adult offspring versus control (CON) sheep. The proportion of nuclear AT_1 receptors sensitive to losartan (LOS) was 2-fold higher [67 ± 6% vs. 27 ± 9%, $p < 0.01$] in BMX compared to control. In contrast, the proportion of AT_2 sites was only one-third that of controls (BMX: 25 ± 11% vs. CON: 78 ± 4%, $p < 0.01$) with a similar reduction in sites sensitive to the Ang-(1-7) antagonist D-Ala^7-Ang-(1-7) with BMX exposure. Functional studies revealed that Ang II stimulated ROS to a greater extent in BMX than control sheep (16 ± 3% vs. 6 ± 4%; $P<0.05$); however NO production to Ang II was attenuated in BMX (26 ± 7% vs. 82 ± 14%; $P<0.05$). BMX-exposure was also associated with a reduction in the Ang-(1-7) NO response [75 ± 8% vs. 131 ± 26%; $P<0.05$]. We conclude that altered expression of angiotensin receptor subtypes may be one mechanism whereby functional changes in NO- and ROS-dependent signaling pathways may favor the sustained increase in blood pressure evident in fetal programming.

Keywords

Angiotensin; receptors; kidney; fetal programming; hypertension

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INTRODUCTION

Glucocorticoids have an important influence on cell maturation and differentiation, particularly in the developing lung[1]. Indeed, the treatment of mothers in immediate jeopardy of preterm delivery with synthetic glucocorticoids enhances fetal lung maturation and lessens respiratory distress syndrome, as well as substantially reduces neonatal mortality and morbidity; glucocorticoid administration is now standard care for women with preterm labor before 35 weeks gestation. However, a single course of antenatal corticosteroid (ANCS) is associated with higher blood pressure in adolescence and decreased insulin sensitivity and renal function in adulthood[2, 3].

Experimental studies demonstrate that exposure of the fetus to elevated glucocorticoids during the critical period of gestation results in the development of elevated blood pressure once the offspring reaches adulthood[4]. In a well-characterized model of fetal programming, adult offspring of sheep exposed antenatally to the glucocorticoid betamethasone (BMX) exhibit increased mean arterial pressure (MAP) that is associated with reduced baroreflex sensitivity and an attenuated capacity to excrete sodium[5–7]. Furthermore, steroid-induced programming may selectively influence the enzymatic components of the renin-angiotensin system (RAS) such that the ratio of ACE to ACE2 is increased in the circulation and kidney favoring the Ang II-AT_1 receptor axis[8, 9]. In this regard, sensitivity to Ang II and other stressors is enhanced in the offspring of glucocorticoid-exposed mothers[9–13]. Indeed, acute treatment with an AT_1 receptor antagonist lowers blood pressure in adult exposed sheep at a therapeutic dose that does not influence pressure in the non-exposed adults[5].

We recently reported the increased expression of the angiotensin receptor subtype AT_1, and a concomitant decrease in the AT_2 subtype within the kidney cortex of older (3–5 years) versus younger adult (1.5 years) sheep[14]. This altered receptor ratio was associated with an increased response in the Ang II-dependent release of reactive oxygen species (ROS) that was abolished by an AT_1 receptor antagonist[14]. In lieu of the functional role of glucocorticoids to influence tissue maturation, we hypothesize that antenatal exposure to glucocorticoids may influence RAS receptors to promote the development and/or progression of high blood pressure associated with fetal programming. The present studies investigated the impact of antenatal BMX administration on the expression and function of angiotensin receptors within the cortical and medullary areas of the adult kidney from control and exposed sheep.

MATERIALS AND METHODS

Animals

Pregnant ewes were administered two intramuscular doses of 0.17 mg/kg betamethasone acetate [BMX] or saline [control] at days 80 and 81 of gestation 24 hours apart[5]. Offspring were delivered at full term, farm raised and transferred to the Wake Forest University School of Medicine animal facility at 1.0–1.5 years of age. Animals were fed a normal diet with access to water *ad libitum*, and maintained on a 12:12 hour light-dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee at Wake Forest University School of Medicine.

Blood Pressure Measurements

Sheep were anesthetized with ketamine and isoflurane followed by catheterization into the femoral artery and vein for blood pressure measurements[5]. After at least 5 days recovery, MAP and heart rate (HR) were recorded in conscious animals and digitized with Acknowledge software (BIOPAC 3.8.1, Santa Barbara, CA). Blood pressure variability (BPV) was measured by power of the spectral density of systolic arterial pressure in the low-

frequency range (LF_{SAP}) in normalized units using the Nevrokard BRS software (Nevrokard BRS, Medistar, Ljubljana, Slovenia)[5].

Tissue Preparation

The kidneys tissues were obtained from adult mixed - breed sheep anesthetized with ketamine and isoflurane. Kidneys were dissected in ice-cold saline into renal cortex and medulla and either immediately frozen on dry ice and stored at −80°C or processed at 4°C for isolation of nuclei.

Isolation of renal nuclei and plasma membrane

Nuclei and plasma membrane fractions were prepared as described previously[14–16]. Fresh or frozen tissue (~0.500 gm) was homogenized in buffer containing 25 mmol/L KCl, 5 mmol/L $MgCl_2$, 20 mmol/L Tricine-KOH and 25 mmol/L sucrose (pH 7.8). The homogenate was passed through a 100-μm mesh-filter and centrifuged twice at 1,000 × g (4°C) for 10 min to obtain the nuclear fraction. The resultant supernatant was centrifuged at 25,000 × g for 20 min (4°C), yielding the plasma membrane fraction. The pellet from the nuclear fraction was re-suspended in 20% OptiPrep solution (Accurate Chemical and Scientific, Westbury, NY) and layered on a discontinuous density gradient column of 25%, 30%, and 35% OptiPrep solution. Each gradient was topped with 10% OptiPrep solution and centrifuged at 10,000 × g for 20 min (4°C). The enriched fraction of isolated nuclei was recovered at the 30–35% layer interface.

Angiotensin binding studies

Angiotensin binding sites were determined with the radioligand ^{125}I-[Sar^1, Thr^8] Ang II (^{125}I-Sarthran) in the presence of losartan, PD123319, D-Ala^7-Ang-(1-7) [A779 or D-Ala] or non-labeled Sarthran as previously described[14].

Reactive Oxygen Species Production

The production of reactive oxygen species (ROS) was measured in isolated renal cortical nuclei using the fluorescence dye, 5-(and-6)-chloromethy1–2′,7′-dichlorodihydrofluorescein diacetate-acetyl ester (DCF; 20 μg/ml) (Molecular Probes, Eugene OR) as described[16]. The NADPH oxidase inhibitor DPI (1 mmol/L) abolished the Ang II response.

Nitric Oxide Production

Production of NO in isolated cortical nuclei was assessed with the fluorescence dye, 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate (DAF; 5μg/ml; Molecular Probes, Invitrogen)[14]. The NOS inhibitor L-NAME (1 mmol/L) abolished the Ang II and Ang-(1-7) responses.

Determination of ACE, ACE2 and neprilysin activities

Peptidase activities were determined in isolated cortical nuclei as described[15]. One unit (U) of activity was defined as one fmol product per mg protein per minute of incubation at 37°C.

Statistical analysis

Data are represented as means ± SEM. Paired or unpaired Student's t-test, one way ANOVA with Tukey's multiple comparison post-hoc and liner regression analysis were performed using GraphPad Prism 5.0 plotting and statistical software.

RESULTS

The MAP was significantly higher in adult sheep exposed antenatally to BMX, as compared to control animals that received saline (98 ± 3 versus 79 ± 2 mmHg; $p<0.05$; Figure 1A). Although the corresponding HR was not different between the BMX-exposed and control sheep (Figure 1B), blood pressure variability ($\%LF_{SAP}$) was significantly higher in the exposed sheep (Figure 1C). Body weights for the two groups of sheep were not significantly different.

The relative proportions of Ang II receptor subtypes were determined for both nuclear and plasma membrane fractions from the renal cortex and medulla of control and BMX-exposed sheep by competition for ^{125}I-Sarthran binding with selective isotype antagonists. As shown in Figure 2A, the AT_1 receptor antagonist losartan competed for a greater percentage of Sarthran binding in both the nuclear and plasma membrane fractions from the BMX-exposed sheep compared to the control animals. Competition by the AT_2 antagonist PD123319 in the renal cortex was lower in both nuclear and plasma membrane fractions of BMX-exposed than control animals (Figure 2B). Similar to the PD123319, the Ang-(1-7) antagonist D-Ala competed to a lesser degree in the cortical nuclei of the BMX-exposed sheep (Figure 2C). In contrast, the AT_1 receptor was the predominant subtype in either nuclei or plasma membrane fractions of the renal medulla of control sheep (Figure 3A). Moreover, the extent of competition by losartan, PD123319 or D-Ala in renal medullary tissue was not altered in BMX-exposed sheep (Figure 3, panels A–C).

We next determined whether functional differences accompanied the alterations in Ang receptor subtypes in the cortical nuclei from BMX-exposed sheep. Generation of ROS (an AT_1- receptor mediated event) was assessed in response to Ang II in freshly isolated nuclei from the renal cortex. Ang II (1 nmol/L) elicited a 2-fold greater increase in ROS in cortical nuclei of BMX-exposed animals than the control animals (Figure 4). The ROS response was significantly reduced in nuclei from BMX-exposed animals pre-incubated with the AT_1 receptor antagonist losartan. In contrast, the AT_2 receptor antagonist PD123319 more than doubled the ROS response to Ang II in cortical nuclei of the BMX-exposed sheep as compared to Ang II alone (Figure 4). Renal nuclei from control animals that were pre-incubated with losartan showed a small reduction in the low levels of ROS produced following stimulation with Ang II. Similar to BMX-exposed sheep, blockade of the AT_2 receptor further increased ROS production in nuclei of control sheep. Treatment with the Ang-(1-7) antagonist D-Ala did not influence the Ang II response in the cortical nuclei from either control or the BMX-exposed sheep (Figure 4). Although not shown, the NADPH oxidase inhibitor DPI abolished the Ang II response consistent with previous data in the kidney of non-exposed sheep and rat[16, 17].

Previous studies in control sheep revealed that both AT_2 and Ang-(1-7) receptors are functionally coupled to NO formation[14, 15]. As shown in Figure 5, NO production in response to Ang II was significantly lower in cortical nuclei from BMX-exposed animals compared to the non-exposed sheep (Control). Blockade of the AT_1 receptor with losartan did not significantly influence NO formation in either the control or BMX-exposed animals; however, pretreatment of nuclei with the AT_2 antagonist PD123319 abolished Ang II-stimulated NO in both groups. The NOS inhibitor L-NAME abolished the NO response to Ang II (data not shown). In addition, co-incubation with the Ang-(1-7) antagonist DAla significantly attenuated the Ang II response in the Control and BMX-exposed animals (Figure 5). As shown in Figure 6, the NO response to Ang-(1-7) [1 nmol/L] was also attenuated in renal cortical nuclei from the BMX-exposed sheep. The D-Ala antagonist abolished the Ang-(1-7) response in both Control and BMX-treated groups; however, neither losartan nor PD123319 influenced NO formation to Ang-(1-7). The Ang-(1-7)

response was abolished by the NOS inhibitor L-NAME (data not shown) consistent with previous results in non-exposed sheep[15].

In lieu of the functional changes for the three Ang receptors following BMX exposure, correlation analyses for the ROS or NO response and changes in mean arterial pressure were performed. As shown in Figure 7A, the ROS response to Ang II was positively correlated to blood pressure ($r = 0.7360$) among the non-exposed and BMX-exposed sheep. In contrast, the NO responses to both Ang II ($r = -0.842$) and Ang-(1-7) ($r = -0.871$) were negatively correlated to blood pressure in the two groups (Figures 7B and 7C, respectively).

Finally, we determined whether differences in the functional response were attributable to alterations in intracellular peptide metabolism. Although ACE, ACE2 and neprilysin were evident on isolated nuclei, their activity levels were similar between the non-exposed and the BMX-exposed animals for ACE [496 ± 111 vs. 388 ± 70 U], ACE2 [161 ± 54 vs. 172 ± 54 U] and neprilysin [297 ± 90 vs. 245 ± 3 U; n = 3 males per group], respectively. Assessment of non-ACE dependent pathways for Ang II (absence of chymostatin) did not reveal intracellular conversion of Ang I to Ang II in either control or treated animals (data not shown).

DISCUSSION

Glucocorticoids are widely administered to mothers who are at risk for pre-term delivery to facilitate the maturation of pulmonary function of the developing fetus. However, exposure to excess endogenous or exogenous glucocorticoids during the perinatal period may "program" the mal-development of the brain and kidney of the offspring for metabolic and cardiovascular disease in adulthood[2–4, 18–20]. We characterized the effects of antenatal exposure to the synthetic glucocorticoid BMX during the 80th day of gestation that corresponds to the peak period of nephrogenesis, as well as the time synthetic glucocorticoids are routinely administered to expectant mothers in jeopardy of premature delivery. The present results find a sustained increase in blood pressure in the exposed sheep and confirm previous findings of a ~15% increase in pressure and blood pressure variability of adult sheep exposed antenatally to BMX[5,8]. The AT_1 receptor antagonist candesartan normalized blood pressure in exposed sheep but did not change pressure in the control animals indicating activation of the Ang II-AT_1 receptor axis in the adult animals exposed to glucocorticoids[5, 8]. Indeed, the current results demonstrate an increase in the ratio of AT_1 to AT_2 receptors within the nuclear and plasma membrane compartments of the renal cortex from the exposed animals. The altered binding profile in the glucocorticoid exposed animals was functionally associated with an enhanced stimulation of ROS by Ang II via AT_1 receptors and a reduction in the AT2 dependent generation of NO. Moreover, the generation of NO by Ang-(1-7) was attenuated in the nuclear fraction isolated from the cortex of the BMX exposed sheep. We did not find changes in angiotensin receptor subtypes within the renal medulla nor intracellular alterations in the peptidases ACE, ACE2, or neprilysin of BMX-treated sheep suggesting selective effects of antenatal steroid exposure on cortical angiotensin receptors. The functional responses for Ang II and Ang-(1-7) were correlated to blood pressure among the non-exposed and steroid-exposed sheep.

We previously reported that Ang II stimulates ROS through the AT_1 receptor within renal nuclei from both rat and sheep[16, 17]. Furthermore, this effect was markedly pronounced in nuclei isolated from older adult sheep which exhibited a greater proportion of AT_1 sites as compared to young adult sheep[16]. The current study revealed that the Ang II-dependent ROS response was 2-fold higher in younger adult sheep following antenatal BMX exposure. Our data support earlier findings by Roghair and colleagues that report an enhanced constriction of coronary vessels by Ang II and increased AT_1 protein expression, as well as

an exaggerated ROS response in glucocorticoid-exposed sheep[21, 22]. The ROS response to Ang II in the present study was reduced with losartan, but exacerbated by PD123319 suggesting that the AT receptor may counterbalance the actions of an activated Ang II-AT_1 receptor pathway[23]. The enhanced response may reflect a reduction in the AT_2-dependent signaling pathways that would normally attenuate Ang II-AT_1 receptor-coupled intracellular events[23] This does not necessarily imply that the AT_1 receptor also exerts an inhibitory influence on AT2 receptor-dependent events within nuclei since losartan did not exacerbate the Ang II-AT_2 receptor pathway for NO. Nonetheless, an increased ratio of AT_1 to AT_2 receptors is consistent with the more pronounced effects of Ang II on sodium reabsorption and renal vascular resistance in the adult sheep following antenatal BMX exposure[11]. Moreover, the reduction in the proportion and functional response of the AT_2 receptor also supports the findings of reduced AT_2 mRNA and/or levels protein levels in the offspring of protein-restricted rats, a rodent model of fetal programming that exhibits higher glucocorticoid levels *in utero*[24-26].

In addition to alterations in the AT_1 to AT_2 receptor ratio, the NO response to Ang-(1-7) was attenuated in the kidney of the exposed sheep. The extent of D-Ala competition for Sarthran binding in the renal cortex was significantly lower in the BMX-exposed group as well. The stimulation of NO by Ang-(1-7) was abolished by D-Ala but not losartan or PD123319. These data suggest that Ang-(1-7)/Mas receptor may also be downregulated by antenatal glucocorticoid exposure. We did not find differences in the intracellular complement of peptidases that influence Ang II and Ang-(1-7) expression; however, it is possible that signaling events downstream from the Ang-(1-7) receptor may be altered in the BMX-exposed sheep. Several reports suggest that Ang-(1-7)-dependent activation of intracellular phosphatases mitigate the actions of the Ang II-AT_1 receptor pathway in proximal tubules and other cell types[27–29], although the nuclear signaling events in the current study, as well as the overall cellular pathways in fetal programming remained to be elucidated. Nonetheless, a reduction in Ang-(1-7)/NO tone supports previous findings that BMX exposure was associated with an attenuated natriuretic and diuretic response to exogenous Ang-(1-7), as well as the overall natriuretic actions of the peptide on the proximal tubule[12,30–32]. Indeed, in control sheep, the Mas receptor protein was evident on proximal tubules, thick ascending limb and collecting ducts of the kidney that are key sites for sodium reabsorption[15]. However, further studies are necessary to determine the cell types within the kidney that reflect the alterations in receptor subtype and response following BMX exposure. We note that the Ang-(1-7) antagonist D-Ala blocked the Ang II-dependent NO response (Figure 4). PD123319 did not inhibit NO synthesis by Ang-(1-7) (Figures 5), therefore, it is unlikely that the Ang II response reflects the conversion to Ang-(1-7) on nuclei by ACE2 and more feasible that the concentration of the D-Ala employed in our studies may interact with the AT_2 receptor.

Emerging evidence reveals that the kidney expresses an intracellular system in which receptors localized within distinct cellular organelle mediate the actions of Ang II and Ang-(1-7)[14–16, 33–35]. There is a high density of intracellular AT_1 sites localized to the nuclear fraction in both the cortical and medullary areas of the rat kidney[33–35]. We also find nuclear AT_1 sites in the cortex of sheep kidney; however, functional AT_2 and Ang-(1-7)/Mas receptors are evident in cortical nuclei as well[14–16]. Intracellular alterations in the ratio of AT_1 to AT2 and/or Ang-(1-7) receptors may influence gene expression that ultimately contributes to programming mechanisms and a sustained increase in blood pressure following glucocorticoid exposure. Zhuo and colleagues report that Ang II via the AT_1receptor directly stimulates mRNA transcripts for the sodium hydrogen exchanger (NHE), MCP-1 and TGF-β in isolated cortical nuclei[35]. Their preliminary studies also show that intracellular expression of non-secreted Ang II in the proximal tubule increases blood pressure that is abrogated by losartan treatment[36]. Nattel and colleagues find that Ang II

increases the mRNA expression of NF*k*B to a greater extent in isolated nuclei from cardiomyocytes than in intact cells[37]. Clearly, the upregulation of sodium transporters such as NHE or the enhanced expression of inflammatory molecules may contribute to renal dysfunction and an elevation in blood pressure; however, whether these mechanisms are operative in this model of programming remains to be established.

Finally, we previously reported that BMX exposure induces a similar increase in blood pressure in female and male sheep at 6 and 36 months of age, as well as a comparable reduction (~25%) in the number of glomeruli[12, 38]. The existing literature on sex differences in fetal programming is somewhat equivocal regarding the extent that females exhibit cardiovascular protection which may reflect species differences, as well as the timing and nature of the programming event[21, 22, 38–42]. The current study did not distinguish sex specific alterations in receptor expression or function due to the low number of females in the BMX-exposed group. We have noted sex differences in sodium excretion and natriuretic responses to exogenous Ang-(1-7) following an acute sodium load in control and exposed sheep that vary with age[11, 12]. It is entirely possible that different, although as of yet undefined mechanisms contribute to the increase in blood pressure in male and female sheep exposed to antenatal glucocorticoids; additional studies are necessary to identify these potential mechanisms. Additional studies are also required to determine whether alterations to intracellular angiotensin receptors precede the changes in blood pressure or contribute to the progression of fetal programmed hypertension. Moreover, at this point it is not evident as to the underlying mechanisms whereby acute fetal BMX exposure alters the relative proportion of the three receptor subtypes within the renal cortex. Finally, although the AT_1 site was the predominant subtype in the renal medulla of both control and BMX-exposed sheep, it is possible that BMX exposure may also influence signaling pathways downstream from the AT_1 receptor, particularly given the importance of ROS and NO in medullary function[43].

PERSPECTIVES

Insults of various types occurring during the critical period of fetal development may have prolonged effects on the offspring in adulthood. Antenatal exposure to excess endogenous or exogenous glucocorticoids, particularly those administered to mothers at risk for pre-term delivery, may predispose the offspring to metabolic and cardiovascular disease including hypertension and renal injury. The effects of steroid exposure on the kidney and kidney development may induce mechanisms that lead to oxidative stress through increased ROS and concomitant decreases in NO. Indeed, steroid-induced programming events may promote a state of accelerated aging that encompasses alterations in multiple angiotensin receptors and their requisite signaling pathways[44]. Thus, potential therapeutic approaches in “fetal programmed” hypertension should consider targeting all three receptor subtypes to re-set the balance of both the extracellular and intracellular RAS.

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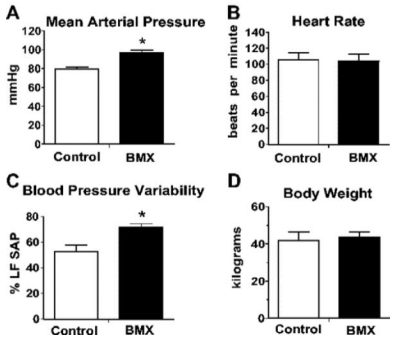

Figure 1.

Influence of antenatal betamethasone (BMX) exposure on mean arterial pressure (A); heart rate (B); blood pressure variability (C); and body weight (D) in adult sheep. Blood pressure variability was determined by power of the spectral density of systolic arterial pressure in the low-frequency range (LF_{SAP}) in normalized units (nu) in non-exposed (Control) and BMX-exposed sheep. Data are 6 males and 2 females per group. Values are means ± SEM; *$P < 0.05$ vs. control.

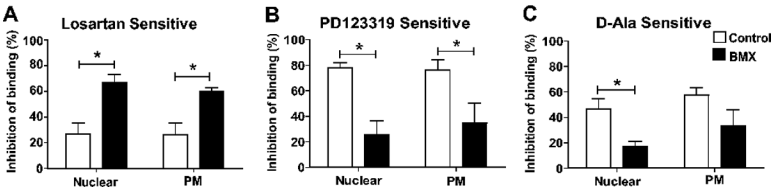

Figure 2.

Angiotensin receptor subtypes in nuclear and plasma membranes (PM) fractions from renal cortex of non-exposed (Control) and betamethasone-exposed (BMX) sheep. Competition binding was performed with 0.5 nmol/L ^{125}I-Sarthran and 10 μmol/L of losartan (A); PD123319 (B); or [D-Ala^{7}]-Ang-(1-7) [C, D-Ala]. Data are expressed as means ± SEM; n = 5 per group (3 males, 2 females) except for D-Ala/PM-BMX (n = 2 males); *p <0.01 vs. Control.

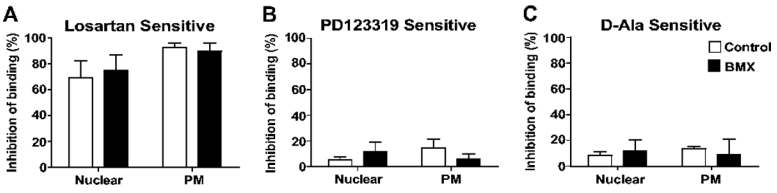

Figure 3.

Angiotensin receptor subtypes in nuclear and plasma membranes (PM) fractions of from renal medulla of non-exposed (Control) and betamethasone-exposed (BMX) sheep. Competition binding was performed with 0.5 nmol/L ^{125}I-Sarthran and 10 μmol/L of losartan (A); PD123319 (B); or [D-Ala^{7}]-Ang-(1-7) [C, D-Ala]. Data are expressed as means ± SEM; n = 5 per group (3 males, 2 females) except for D-Ala/PM-BMX (n = 2 males).

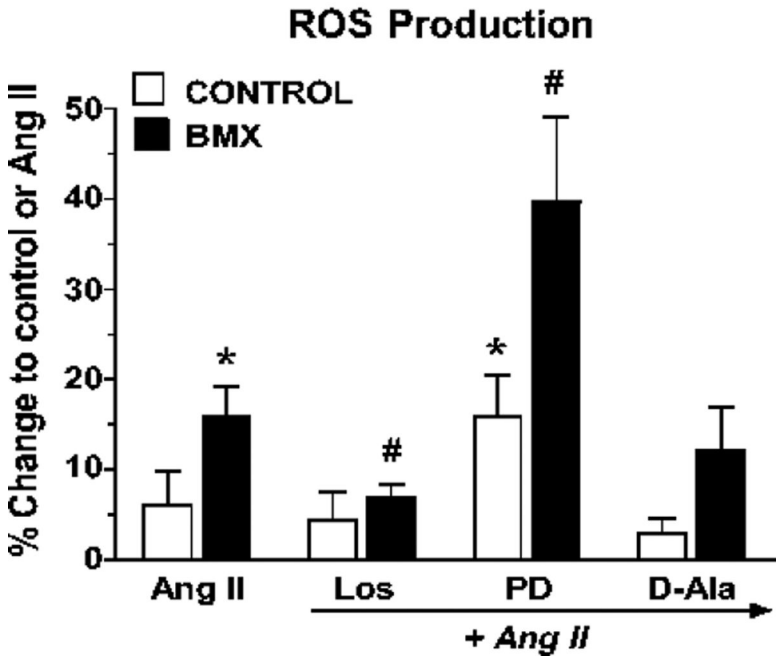

Figure 4.

Angiotensin II (Ang II) stimulates reactive oxygen species (ROS) in renal cortical nuclei from non-exposed (Control) and betamethasone-exposed (BMX) sheep. Cortical nuclei were freshly isolated by gradient separation and pre-incubated with the fluorescent dye, dichlorofluorescein (DCF). Nuclei were stimulated with Ang II (1 nmol/L) in the presence (1 μmol/L final concentration) of losartan (Los), PD123319 (PD) or [D-Ala^7]-Ang-(1-7) [D-Ala]. Data are the means ± SEM; *p< 0.05 vs. Ang II - Control; #p < 0.05 vs. Ang II - BMX. Control group is n = 11 (8 male, 3 female); BMX group is n = 8 (6 male, 2 female).

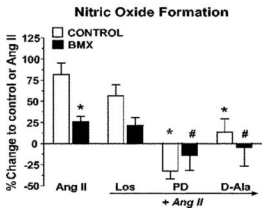

Figure 5.

Angiotensin II (Ang II) stimulates nitric oxide (NO) in renal cortical nuclei from non-exposed (Control) and betamethasone-exposed (BMX) sheep. Cortical nuclei were freshly isolated by gradient separation and pre-incubated with the selective NO fluorescence detector, DAF. Nuclei were stimulated with Ang II (1 nmol/L) in the presence (1 μmol/L final concentration) of losartan (Los), PD123319 (PD) or [D-Ala^7]-Ang-(1-7) [D-Ala]. Data are the means ± SEM; *p< 0.05 vs. Ang II - Control; #p < 0.05 vs. Ang II - BMX. Control group is n = 11 (8 male, 3 female); BMX group is n = 8 (6 male, 2 female).

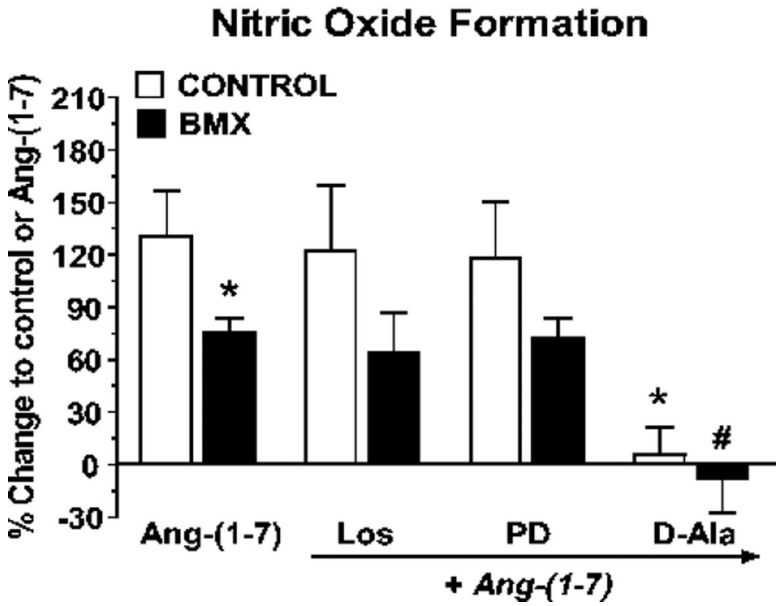

Figure 6.

Angiotensin-(1-7) [Ang-(1-7)] stimulates nitric oxide (NO) in renal cortical nuclei from non-exposed (Control) and betamethasone-exposed (BMX) sheep. Cortical nuclei were freshly isolated by gradient separation and pre-incubated with the selective NO fluorescence detector, DAF. Nuclei were stimulated with Ang II (1 nmol/L) in the presence (1 μmol/L final concentration) of losartan (Los), PD123319 (PD) or [D-Ala^7]-Ang-(1-7) [D-Ala]. Data are the means + SEM; *p< 0.05 vs. Ang-(1-7) -Control; #p < 0.05 vs. Ang-(1-7) - BMX. Control group is n = 6 (4 male, 2 female); BMX group is n = 5 (4 male, 1 female).

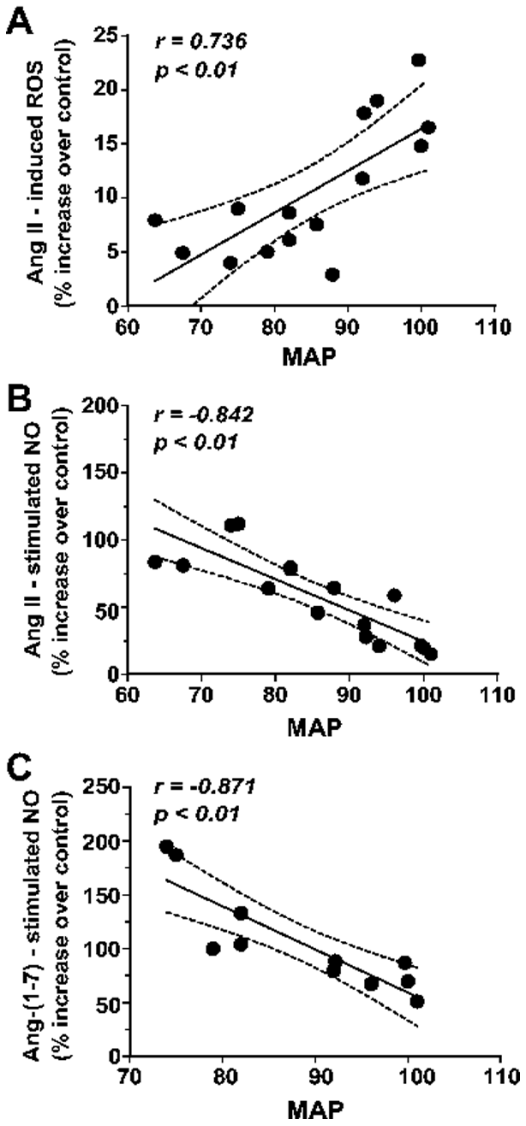

Figure 7.

Correlation analyses reveal a positive association of the angiotensin II-reactive oxygen species [Ang II-ROS] response and mean arterial blood pressure (MAP) (A) but negative associations for the Ang II-nitric oxide [Ang II-NO] (B) and angiotensin-(1-7)-NO [Ang-(1-7)-NO] (C) responses with MAP in non-exposed and betamethasone-exposed sheep.