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Prenatal Lipopolysaccharide Exposure Results in Dysfunction of the Renal Dopamine D₁ Receptor in Offspring

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Abstract

Adverse environment in early life can modulate the adult phenotype, including blood pressure. Lipopolysaccharide (LPS) exposure *in utero* results in increased blood pressure in the offspring, but the exact mechanisms are not clear. Studies have shown that the renal dopamine D₁ receptor (D₁R) plays an important role in maintaining sodium homeostasis and normal blood pressure; dysfunction of D₁R is associated with oxidative stress and hypertension. In this study, we determined if dysfunction of the renal D₁R is involved in fetal-programmed hypertension, and if oxidative stress contributes to this process. Pregnant Sprague-Dawley (SD) rats were intraperitoneally injected with LPS (0.79 mg/kg) or saline at gestation days 8, 10, and 12. As compared with saline-injected (control) dams, offspring of LPS-treated dams had increased blood pressure, decreased renal sodium excretion, and increased markers of oxidative stress. In addition, offspring of LPS-treated dams had decreased renal D₁R expression, increased D₁R phosphorylation and G protein-coupled receptor kinase type 2 (GRK2) and type 4 (GRK4) protein expression and impaired D₁R-mediated natriuresis and diuresis. All of the findings in the offspring of LPS-treated dams were normalized after treatment with TEMPOL, an oxygen free radical scavenger. In conclusion, prenatal LPS exposure, via an increase in oxidative stress, impairs renal D₁R function and leads to hypertension in the offspring. Normalization of renal D₁R function by amelioration of oxidative stress may be a therapeutic target of fetal programming of hypertension.

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Disclosure

There are no conflicts of interest, financial or otherwise.

Keywords

fetal programming; hypertension; lipopolysaccharide; D₁ dopamine receptor; G protein-coupled receptor kinase; TEMPOL; oxidative stress

Introduction

Hypertension, an important cardiovascular risk factor and a major contributor to end-stage renal disease, is a heterogeneous disorder that is probably caused by an interaction of genetic and environmental factors [1]. In recent years, a large number of experiments have revealed that fetal programming (also known as developmental origins of adult diseases), is another important factor contributing to the development of hypertension [2, 3]. Barker and colleagues have proposed that adverse environmental stimuli, experienced during a critical period of development *in utero*, could induce long-term structural and functional effects in the developing organism [4, 5].

Maternal infection and inflammation are common events during pregnancy, and a large number of studies support the relationship between infection and adverse pregnancy outcomes [6–9]. Intrauterine infection is considered as one of the major maternal insults during pregnancy [10]. Lipopolysaccharide (LPS) is a toxic component of cell walls of gram-negative bacteria that is present in the digestive tracts of humans and animals [11]. Peripheral administration of LPS from *Escherichia coli* is a well-characterized model of sepsis in rodents [12]. Maternal exposure to LPS alters proinflammatory cytokine levels, including IL-1, IL6, and TNF- α , in the placenta, amniotic fluid, and fetal brain, that may have a significant impact on fetal development [13]. Studies have shown that prenatal exposure to LPS results in increased blood pressure in the offspring that is associated with alterations in the renin-angiotensin system in the kidney and increased circulating leptin which can increase sympathetic nerve activity [14, 15]. However, hypertension is caused not only by increased activity of pro-hypertensive factors, such as the renin-angiotensin and sympathetic nervous systems, but also by decreased activity of anti-hypertensive mechanisms, such as the renal dopaminergic system [16].

Dopamine, produced in the kidney independent of renal nerves, is now recognized to serve an important role in the regulation of sodium balance and blood pressure. Dopamine receptors are classified into D₁- (D₁R, D₅R) and D₂-like (D₂R, D₃R and D₄R) subtypes based on their structure and pharmacology [16–18]. Under euvolemic conditions and moderate volume expansion, dopamine, especially via D₁-like receptors, acts to increase sodium excretion and keep the blood pressure in the normal range. Abnormal responses to dopamine and D₁-like receptor function have been implicated in hypertensive patients and rodent models of genetic and salt-sensitive hypertension [16–21]. Previous studies have found that dysfunction of D₁R can be the result of its hyper-phosphorylation caused by G protein-coupled receptor kinase type 2 (GRK2) and GRK4 [16, 19, 22], that can be linked to increased oxidative stress [23–28]. Antioxidant supplementation with TEMPOL, a water-soluble, paramagnetic nitroxyl radical with superoxide dismutase (SOD) mimetic activity decreases oxidative stress, restores D₁R signaling, and lowers blood pressure [26–28]. Thus,

we investigated whether or not dysfunction of renal D₁R signaling is involved in the fetal programming of hypertension and whether or not inhibition of reactive oxygen species (ROS) production would ameliorate the renal D₁R dysfunction and normalize blood pressure.

Materials and Methods

1. Animals and treatment

The experimental protocols were approved by The Third Military Medical University Animal Care and Use Committee. Virgin Sprague-Dawley (SD) rats (250-260g) were purchased from the Animal Centre of The Third Military Medical University (Chongqing, China). All rats were intraperitoneally administered with LPS (0.79mg/kg in 0.5 ml saline, *Escherichia coli*, Sigma, St Louis, MO, USA, L8274,) or saline (0.5ml) on gestation days 8, 10, and 12 (n=10 in each group), and were housed individually throughout pregnancy until delivery. The pups were weaned at 4 weeks of age to regular rat chow.

The blood pressure was measured in conscious rats at 5 weeks, and 3, 6, 9, and 12 months of age using the tail-cuff method (ML125, PowerLab, AD Instruments, Castle Hill, Australia). Urine was collected in metabolic cages, and 24-hour urine volume and sodium excretion were measured. At 12 weeks of age, male offspring of LPS- and saline-treated dams were randomly assigned into two groups: one group that drank tap water served as control, and the other group drank tap water with 1.0 mmol/L TEMPOL, that was changed two times a day for 3-4 weeks.

Renal Histology—Histological analysis was performed on kidney samples taken at 12 months of age from the offspring of vehicle- (control) and LPS-treated dams. The tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 4- μ m-thick sagittal sections. The sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome and examined for glomerulosclerosis, interstitial fibrosis, and tubular atrophy.

Surgical procedures and experimental protocol for renal function studies—The surgical procedures and surgical interventions were performed as reported [29]. The rats were anesthetized with pentobarbital sodium (50 mg/kg body wt), placed on a heated table to maintain rectal temperature at ~37°C, and tracheotomized (PE-240). Anesthesia was maintained by the infusion of pentobarbital at 0.8 mg/100g body wt per hour. The left carotid artery was catheterized with PE-50 tubing, which was connected to a pressure transducer (Cardiomax II; Columbus Instruments, Columbus, OH) for blood pressure measurement. The left external jugular vein was catheterized for fluid administration. A midline incision was performed; the left and right ureters were catheterized with PE-10 tubing for urine collection. The right suprarenal artery was catheterized (PE-10, heat stretched to 180 μ m) for vehicle (saline) or fenoldopam infusion. The duration of the surgical procedures was about 60 min. During the experiment, vehicle (saline) or fenoldopam was infused at a rate of 40 μ l/h. Fluid losses throughout the experiment were replaced intravenously with 5% albumin at 1% body wt over 30 min.

After a 60-min stabilization period following the surgery, seven consecutive 40-min urine samples were collected: C1 and C2 (averaged as basal period), D1, D2, and D3 (averaged as fenoldopam period), and R1 and R2 (averaged as recovery period). During the basal and recovery periods, only saline alone was infused. During the fenoldopam period, fenoldopam (1.0 µg/kg/min in saline, Tocris Bioscience, 1659), a D₁-like receptor agonist, was infused. Urine was collected every 40 min. Blood samples (300 µl, replaced intravenously by equal amount of 5% albumin) were collected at the end of basal, fenoldopam, and recovery periods. Plasma was separated from blood particulate matter by centrifuging the blood samples at 2,500 g for 15 min at 4°C. Urine and plasma samples were stored at -80°C until use.

Blood and urine analysis—The urine sodium concentration was analyzed by a flame photometer 480 (Ciba Corning Diagnostics, Norwood, MA). Creatinine and urea nitrogen levels were measured with commercially available kits (Nanjing Jianchen Bioengineering Institute, Nanjing, China). Random blood glucose was measured with a glucose analyzer (Roche, Indianapolis, IN).

Biochemical markers of oxidative stress—To assess oxidative stress in the whole body, the lipid peroxidation product, malondialdehyde (MDA) in the urine was quantified using a commercially available kit (Nanjing Jianchen Bioengineering Institute, Nanjing, China). To assess antioxidants, plasma and renal samples from rats were used to measure superoxide dismutase activity using an SOD Assay Kit (Dojindo Laboratories, Kumamoto, Japan), and glutathione (GSH) levels, using a commercially available kit (Nanjing Jianchen Bioengineering Institute, Nanjing, China) following the manufacturer's instructions.

Preparation of proximal tubular membranes—Proximal tubular membranes were prepared as described previously [28]. Briefly, proximal tubular suspensions were homogenized in buffer (10 mM Tris HCl, 250 mM sucrose, 2 mM PMSF, protease inhibitor cocktail; pH 7.4), and centrifuged at 24,000 g for 25 min at 4°C. The upper fluffy layer of the pellet was resuspended in the homogenization buffer and was considered as the membrane fraction; the supernatant was considered as the cytosolic fraction. Finally, the samples were quickly frozen and stored at -80°C until use.

Immunoblotting of D₁R, GRK2, and GRK4—Equal amounts of proteins (60 µg) were resolved in 10% SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk, in tris-buffered saline (TBS) with 0.05% tween-20, for 90 min at room temperature. Then the membranes were incubated with rabbit polyclonal D₁R (1:500, Millipore, Billerica, MA, AB1765P), rabbit polyclonal GRK4 (1:400, Santa Cruz Biotechnology, CA, SC-13079), rabbit polyclonal GRK2 (1:400, Santa Cruz Biotechnology, SC-562), or rabbit polyclonal GAPDH (1:500, Santa Cruz Biotechnology, SC-25778) antibodies overnight at 4°C. The membrane-bound antibodies were visualized using horseradish peroxidase-conjugated secondary antibodies (1:12,000, Li-Cor Bioscience, 926-32213) and the Odyssey Infrared Imaging System (Li-Cor Bioscience, Bad Homburg, Germany). The densities of the bands were quantified by densitometry using Quantity-One software (Bio-Rad, Hercules, CA), and normalized with GAPDH.

Detection of serine-phosphorylated D₁R—The proximal tubular membranes were incubated with D₁R antibody (2μg, Millipore, Billerica, MA, AB1765P) for 2h followed by protein A/G agarose overnight with rocking at 4°C. The immunoprecipitates were pelleted and washed 3 times with phosphate-buffered saline. Then the pellets were suspended in 2× Laemmli buffer at 95°C for 10 min, and subjected to immunoblotting with phosphoserine antibody (1:400, Immunechem, Burnaby, BC, Canada, ICP9806). Band density of the serine-phosphorylated D₁R was normalized by band density of D₁R. All the bands were quantified by densitometry.

Statistical Analysis—The data are expressed as mean ± SEM. Comparison within groups was made by repeated measures ANOVA (or paired t-test when only 2 groups were compared), and comparison among groups (or t-test when only 2 groups were compared) was made by factorial ANOVA with Holm-Sidak test. A value of P<0.05 was considered significant.

Results

1. Effects of prenatal LPS exposure on blood pressure and renal function in offspring

As shown in Table 1, there were no obvious differences among the four groups, regarding general characteristics, including heart rate, random plasma glucose level, and kidney/weight ratio. However, the offspring of LPS-treated dams were heavier than vehicle-treated (control) dams, consistent with previous reports [15]. Systolic blood pressure measured by tail-cuff method was higher in LPS-treated offspring than vehicle-treated control offspring starting at 5 week of age (Fig. 1A). The difference in systolic blood pressure between the offspring of control- and LPS-treated dams progressively increased with age (Fig. 1B).

There was no obvious difference in the gross structure of the kidneys from 12-month-old offspring of control and LPS-treated dams (Fig. 2). Moreover, the plasma urea nitrogen and creatinine concentrations were not different between the two groups (Table 1). However, 24h urine volume and sodium excretion were lower in 15-week old offspring of LPS-treated dams than the offspring of control dams (Fig. 3).

2. Role of ROS in the fetal programming of hypertension

In vitro and *in vivo* studies have shown that LPS increases inflammation and ROS production [11, 30]. Therefore, we first measured ROS production in the offspring of LPS- and vehicle-treated (control) dams. We found that urine MDA levels were higher while renal and plasma SOD, and renal GSH levels were lower in the offspring of LPS- than vehicle-treated dams (Fig. 4). To determine directly the role of ROS in the fetal programming of hypertension, 12-week-old offspring of LPS-treated dams were treated with TEMPOL, a superoxide dismutase (SOD) mimetic, for 3 weeks. We found that the increase in sodium excretion and normalization of blood pressure by TEMPOL (Fig. 3) were accompanied by a decrease in urine MDA and an increase in plasma and renal SOD, and renal GSH levels (Fig. 4) in the offspring of LPS-treated dams. TEMPOL did not affect these variables in the offspring of vehicle-treated dams.

3. Role of D₁R in the fetal programming of hypertension

As shown in previous reports [31], D₁-like receptor stimulation induced diuresis and natriuresis in Wistar-Kyoto and SD rats. To determine whether or not there is impaired D₁-like receptor function in the offspring of LPS-treated dams, we studied the diuretic and natriuretic effects of fenoldopam, a D₁-like receptor agonist, infused directly into the right renal artery, via the right suprarenal artery. Consistent with previous reports [20, 32], fenoldopam induced diuresis and natriuresis in the offspring of vehicle-treated dams, while the fenoldopam-mediated diuresis and natriuresis were markedly attenuated in the offspring of LPS-treated dams (Fig. 5).

In the current study, we also found that renal D₁R expression (Fig. 6) was lower and renal D₁R serine-phosphorylation (Fig. 7) was greater in the offspring of LPS-treated dams than the offspring of vehicle-treated (control) dams. Moreover, renal GRK2 and GRK4 expressions were also greater in the offspring of LPS- than vehicle-treated dams (Fig. 6), implicating both GRK2 and GRK4 in the dysfunction of renal D₁R in the fetal-programmed hypertension.

As shown in Figs. 3 and 4, TEMPOL treatment normalized the urine flow, urine sodium excretion, blood pressure, and redox status in the offspring of LPS-treated dams. To determine whether or not oxidative stress participated in the decreased renal D₁R expression and increased renal D₁R phosphorylation, and GRK2 and GRK4 expressions observed in the offspring of LPS-treated dams, we studied the effect of TEMPOL on these variables. After TEMPOL treatment for 3 weeks, the decreased renal D₁R expression, and increased renal D₁R phosphorylation and GRK2 and GRK4 expressions were reversed to normal levels in the offspring of LPS-treated dams (Figs. 6 and 7). The impaired diuretic and natriuretic effects of the D₁-like receptor agonist fenoldopam in the offspring of LPS-treated dams were also ameliorated by TEMPOL (Fig 5). There were no effects of TEMPOL in the vehicle-treated (control) dams. These results could be taken to indicate that the high levels of ROS induced by LPS in the offspring of LPS-treated dams is the major factor that led to the increased renal GRK2 and GRK4 expression and impaired D₁R function.

Discussion

Many epidemiological and experimental studies have shown that several adult diseases, including cardiovascular disease, have fetal origins, emphasizing the importance of fetal programming on adult diseases [4, 5, 33–35]. As summarized (Fig. 8), our current study showed that offspring of dams exposed to LPS, to model infection during pregnancy, develop hypertension, accompanied by abnormalities of the renal dopaminergic system.

Hypertension can be programmed by adverse environment *in utero* [4, 5, 33–35]. LPS, an endotoxin from gram-negative bacteria, acts as a potent trigger of oxidative stress and also a disruptor of antioxidant defenses [36]. During infection, people are constantly exposed to low levels of LPS [37]. The placenta is known to generate cytokines in response to infection; human amniochorionic membranes produce IL-1 β , IL-6, and TNF- α in response to LPS *in vitro* [38], and these cytokines generated by the placenta are also likely to gain entry into the fetal circulation [13]. Maternal exposure to the bacterial endotoxin LPS has been used to

study the effects of systemic inflammation during pregnancy [37, 39]. We chose to inject LPS in dams on gestational days 8, 10, and 12 because these periods correspond to the first-to-second trimesters of human pregnancy, one of the main periods of vulnerability of the immune system to environmental insults [40]. The dose of LPS (0.79mg/kg) used in the current study has been reported to induce systemic inflammation with low percentage of fetal anomalies and no or low rate of abortions [41]. Our present study also showed prenatal LPS exposure causes a low percentage of abortion about 10% at this dosage.

A previous study showed that exposure of dams to LPS increased blood pressure that was accompanied by decreased glomerular number and creatinine clearance and increased urinary protein in offspring [14]. However, in our current study, we did not find a significant increase in plasma creatinine in 15-week old offspring of LPS-treated dams. The reason(s) for the differences in renal function between our study and the studies of Hao et al is not clear. Age could be a factor, because creatinine clearance was decreased in 6-month-old offspring of diabetic dams [33]. In our study, we did not evaluate the renal function of the offspring at 6 months of age or beyond. The creatinine clearances were also not consistently decreased in the offspring of LPS-treated dams reported by Hao et al. In one report [14], they showed that maternal exposure to LPS caused a decrease in creatinine clearance rate and glomerular number in the offspring while in another study, the offspring of LPS-treated dams had normal plasma urea and creatinine concentrations [42].

Hypertension is characterized by impaired sodium handling and sodium retention by kidney [43], which appears to be susceptible to the effects of programming in utero [44]. The kidney and its regulation of sodium balance are of primary importance in the regulation of blood pressure, regardless of the initiating factor [45]. Our current study showed that the hypertension and decreased sodium excretion of offspring of LPS-treated dams were accompanied by abnormalities of the renal dopaminergic system. Renal dopamine, independent of renal nerves, plays an important role in maintaining sodium homeostasis and blood pressure regulation, especially during moderately increased sodium intake [16, 19, 46]. Under conditions of moderate sodium excess, the renal dopaminergic system, mainly the D₁R, is responsible for more than 50% of renal sodium excretion [16]. Abnormalities in renal dopamine and D₁R response to an increased sodium load have been implicated in the diminished natriuretic response and increase in blood pressure in hypertensive patients and rodents [16, 18, 19, 24, 29, 31, 46]. Our current study found that the renal D¹R expression is decreased and D¹R phosphorylation is increased, conditions that should lead to impaired D₁R-mediated natriuresis and diuresis in the offspring of LPS-treated dams. We have reported that in spontaneously hypertensive rats, the renal D₁R is hyper-phosphorylated [47]. Studies have shown that increased GRK expression and GRK4 gene variants are involved in the hyperphosphorylation of D₁R, resulting in its desensitization and uncoupling from G protein partners; both GRK2 and GRK4 activities and expression are increased in some hypertensive humans and animal models of hypertension [16, 18, 19, 22–27, 31, 46–54]. Obese Zucker rats are hypertensive and also have decreased renal D₁R expression, increased renal D₁R phosphorylation, defective D₁R and G protein subunit coupling, impaired dopamine inhibition of sodium transport and natriuretic response to D₁-like receptor stimulation and increased expression of renal proximal tubule membrane GRK2 and GRK4 [52]. Consistent with these reports, we also found that GRK2 and GRK4

expressions are increased in renal cortical membranes while renal cortical membrane D₁R expression and D₁-like receptor function are decreased in offspring of LPS-treated dams. Thus, the current studies support the notion that both GRK2 and GRK4 are involved in the impaired function of renal D₁R in hypertension.

Studies indicate that both hypertensive patients and animals have decreased antioxidant capacity and produce excessive amounts of ROS [24–27, 54–57]. Antioxidant treatment could mitigate the production of ROS, and further increase antioxidant capacity, such as SOD and GSH in plasma and tissue. LPS is known to increase maternal serum TNF- α levels [58] and stimulate macrophages to generate ROS [11, 59, 60], and enhance oxidative stress in the placenta and fetus [59]. Therefore, we hypothesized that there may be increased ROS and decreased antioxidant capacity in offspring of LPS-treated dams. Large amount of studies have shown that the impaired intrarenal D₁R signaling in hypertension can cause or be caused by oxidative stress [24–28, 54, 61–64]. Treatment with the superoxide dismutase mimetic TEMPOL has been shown to reduce GRK2 and GRK4 levels and restore D₁R expression and D₁R coupling and function and normalize blood pressure by decreasing oxidative stress in old and obese rats [23, 27, 28, 64]. The long-term (3 weeks) intravenous infusion of TEMPOL also decreases arterial pressure of Dahl salt-sensitive rats on a high sodium intake, reducing their salt-sensitivity, renal production of O₂⁻ and renal damage [56]. Our current study showed high ROS levels and decreased antioxidant capacity (SOD and GSH) in the offspring of LPS-treated dams. The administration of TEMPOL restored ROS to normal state, increased D₁R-mediated natriuresis and diuresis, and lowered the blood pressure of the offspring of LPS-treated dams; TEMPOL also normalized the renal GRK2, GRK4, and D₁R expressions and D₁R phosphorylation.

In summary, our study shows direct evidence supporting the hypothesis of fetal-programmed hypertension, and, for first time, links the dysfunction of renal D₁R to the fetal-programmed hypertension secondary to maternal LPS exposure. Increased oxidative stress, associated with increased GRK2 and GRK4 levels, impairs renal D₁R function, and leads to hypertension. Restoration of D₁R function by antioxidants may be a therapeutic target for fetal-programmed hypertension.

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1. Prenatal LPS exposure leads to increased blood pressure and ROS in offspring.
2. The diuretic and natriuretic effects induced by renal D₁R is decreased in LPS rats.
3. The level of renal GRK2 and GRK4 and serine-phosphorylation of D₁R are increased.
4. Antioxidant TEMPOL could reverse the function of renal D₁R and GRK4 level.
5. Dysfunction of renal D₁R could be fetal origins.

Fig. 1. Effect of prenatal LPS exposure on blood pressure in offspring.

Systolic blood pressure (**A**, SBP) and difference in SBP (**B**) between the offspring of LPS- and vehicle-treated (control) dams. SBP was measured by tail-cuff method in 5 week- and 3-, 6-, 9-, and 12-month old offspring of vehicle- (control) and LPS-treated dams. * $P < 0.05$ versus offspring of vehicle-treated (control) dams, # $P < 0.05$ versus 5 weeks ($n = 6-10$).

Fig. 2. Effect of prenatal LPS exposure on renal histopathology in offspring. Renal histopathology was examined by H&E and Masson's trichrome staining in 12-month-old offspring of vehicle (control)- and LPS-treated dams. Original magnification $\times 200$.

Fig. 3. Effect of TEMPOL on 24h urine volume, 24h urine sodium excretion, and blood pressure in offspring of vehicle (control)- and LPS-treated dams.

24h urine volume (A), 24h urine sodium excretion (B), systolic blood pressure (SBP, C), and mean blood pressure (MBP, D) were measured in 15-week-old offspring of vehicle (control)- and LPS-treated dams after treatment with vehicle or TEMPOL (1.0 mmol/L in drinking water) for 3 weeks. * $P < 0.05$ versus offspring of control dams, # $P < 0.05$ versus offspring of LPS-treated dams ($n = 8$).

Fig. 4. Effect of prenatal LPS exposure on oxidative stress in offspring.

Urine MDA (A), plasma SOD (B), renal SOD (C), and GSH (D) were quantified in 15 week-old offspring of vehicle (control)- and LPS-treated dams after treatment with vehicle or TEMPOL (1.0 mmol/L in drinking water) for 3 weeks. * $P < 0.05$ versus offspring of control dams, # $P < 0.01$ versus offspring of LPS-treated dams ($n = 6$).

Fig. 5. Effect of prenatal LPS exposure on renal D₁R function in offspring.

Urine flow (A), and urine sodium excretion rates (B) were measured in 15 week-old offspring of vehicle (control)- and LPS-treated dams after treatment with vehicle or TEMPOL (1.0 mmol/L in drinking water) for 3 weeks. The D₁-like receptor agonist, fenoldopam (1.0 μg/kg/min) was infused into the right renal artery via the right suprarenal artery of anesthetized rats. Basal: values before fenoldopam administration; Fenoldopam: values during fenoldopam administration; Recovery: values after fenoldopam infusion.

*P<0.05 versus basal control-offspring of control dams, #P<0.05 versus basal-offspring of LPS-treated dams, \$P<0.05 versus other groups in Fenoldopam period (n=8).

Fig. 6. Effect of prenatal LPS exposure on renal GRK2, GRK4, and D₁R protein expressions in offspring.

Renal cortical membrane GRK2 (A), GRK4 (B), and D₁R (C) expressions were quantified by immunoblotting in 15 week-old offspring of vehicle (control)- and LPS-treated dams after treatment with vehicle or TEMPOL (1.0 mmol/L in drinking water) for 3 weeks, *P<0.05 versus offspring of control dams, #P<0.05 versus offspring of LPS-treated dams (n =6).

Fig.7. Effect of prenatal LPS exposure on D₁R serine-phosphorylation in renal cortical membranes in offspring.

Renal cortical membrane D₁R serine-phosphorylation was quantified in 15 week-old offspring of vehicle (control)- and LPS-treated dams after treatment with vehicle or TEMPOL (1.0 mmol/L in drinking water) for 3 weeks. Immunoprecipitated D₁R from renal cortical membranes were immunoblotted for serine-phosphorylated D₁R (top band) and total D₁R expressions (bottom band). The band densities of serine-phosphorylated D₁R were normalized by band densities of total D₁R. *P<0.05 versus others, #P<0.05 versus offspring of LPS-treated dams (n =6).

Fig. 8. Schematic illustration of the effect of maternal infection *in utero*, mimicked by maternal LPS-injection, on renal D₁R function and blood pressure in offspring.

Prenatal LPS exposure, as a model maternal infection, increases renal GRK2 and GRK4 expressions caused by oxidative stress that lead to renal hyperphosphorylation of D₁R, diminished D₁R-mediated diuresis and natriuresis, and ultimately hypertension.

Table 1.

General characteristics

	Control	Control+TEMPOL	LPS	LPS+TEMPOL
Body weight(g)	280±11	276±12	295±10 [*]	283±15
Kidney/weight (g/kg wt)	3.40±0.07	3.38±0.12	3.44±0.10	3.41±0.09
Heart rate (beats/min)	365±16	372±16	367±12	366±17
Random plasma glucose (mmol/L)	7.35±0.9	7.25±0.69	7.57±0.48	7.47±0.46
Plasma creatinine (mmol/L)	31.13±1.79	28.75±4.69	30.38±6.57	32.38±2.25
Plasma urea nitrogen (mmol/L)	6.10±1.56	6.21±1.25	6.03±0.69	6.20±2.03
24h Urine volume(ml/kg wt)	38.8±2.69	41.58±3.18	25.58±2.09 [*]	55.42±4.08 [#]
24h Urine sodium (mmol/kg wt)	0.85±0.05	1.06±0.10	0.52±0.01 [*]	1.25±0.07 [#]

These data were collected in 15 week-old offspring of vehicle (control)- and LPS-treated dams after treatment with vehicle or TEMPOL (1.0 mmol/L in drinking water) for 3 weeks. Results are mean±SE, n=6-8.

* P<0.05 versus Control

P<0.05 versus LPS.