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Effects of inflammatory stimulation during pregnancy on mesenteric vascular function of offspring rats

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Abstract

To evaluate the effects of inflammatory stimulation during pregnancy on the mesenteric vascular function of offspring rats. Twenty-four Sprague-Dawley pregnant rats were randomly divided into a control group and a lipopolysaccharide (LPS) stimulation group that were intraperitoneally injected with normal saline and LPS (0.79 mg/kg) on the 8th, 10th and 12th days of pregnancy respectively. At 8 weeks of age, 12 male pups were randomly selected from each group for subsequent experiments. Their blood pressures at 9, 11, 13 and 15 weeks of age were measured noninvasively at the tail. The responses of mesenteric tertiary blood vessels to 10^{-10}~10^{-5} mol/L angiotensin II (AngII) were observed. AT1R expression in mesenteric vessels was detected by Western blotting. The body weight of offspring rats at the age of 15 W was higher in LPS group than that of control group (P<0.05). No statistically significant differences were found in blood glucose, creatinine and plasma urea nitrogen between the two groups (P>0.05). The systolic blood pressures at 9, 11, 13 and 15 W were (114 ± 11) mmHg, (125 ± 12) mmHg, (132 ± 11) mmHg and (139 ± 11) mmHg respectively in LPS group, which were (101 ± 12) mmHg, (112 ± 11) mmHg, (118 ± 12) mmHg and (121 ± 11) mmHg respectively in control group. The systolic blood pressure of the LPS stimulation group was significantly higher than that of the control group at 9, 11, 13 and 15 W (P<0.05). The maximum contractile response of the mesenteric artery induced by AngII in the LPS group exceeded that of the control group [(8.49 ± 0.32) vs. (3.47 ± 0.29) mN, P<0.05]. When the concentrations of AngII were 10^{-7}, 10^{-6} and 10^{-5} mmol/L, the contractile responses of the mesenteric artery in the LPS group all exceeded those of the control group [(5.99 ± 0.48) vs. (2.21 ± 0.19), (7.10 ± 0.11) vs. (3.02 ± 0.43) and (8.51 ± 0.39) vs. (3.49 ± 0.29) mN respectively, P<0.01]. After pre-incubation with losartan for 30 min, the contractile response to AngII of each group was inhibited, without a significant difference (P>0.05). The relative protein expression of AT1R in the mesenteric arteries of offspring rats was 1.51 ± 0.10 in LPS group and 1.01 ± 0.09 in control group. The protein expression of AT1R in the mesenteric arteries of offspring rats was significantly higher in LPS group than that in control group (P<0.05). Exposure to LPS during pregnancy leads to abnormal AngII-mediated vasoconstriction and increases the vascular resistance in offspring rats, which may be an important reason for the elevation of blood pressure.

Key words: pregnancy; inflammatory stimulation; lipopolysaccharide; mesentery; vascular function

1. Introduction

The number of deaths due to cardiovascular disease is 17.3 million per year,which accounts for 30% of all deaths worldwide. These diseases are related to cardiac or vascular pathological changes [1], commonly including hypertension, coronary heart disease, aneurysm, peripheral vessel obstruction and cardiomyopathy. Therefore, the prevention and treatment of these diseases are

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urgent. Hypertension is one of the most serious diseases that threaten human health. It is well-known that hypertension is an inflammatory disease, so patients have high levels of inflammatory factors such as C-reactive protein, interleukin (IL)-6 and tumor necrosis factor-α [2]. Meanwhile, the levels are positively correlated with blood pressure. Until now, most studies have focused on the prevention and treatment of hypertension in adults. Recent studies have proven that stimulation using early adverse factors caused hypertension in offspring rats [3]. Besides, exposure to lipopolysaccharide (LPS) or zymosan during pregnancy can cause hypertension in adult offspring rats, but the mechanism remains unclear [4]. LPS is a unique chemical component in the outer wall layer of Gram-negative bacteria. With a molecular weight of >10000, it is a complex of lipids and polysaccharides, being extremely harmful to human body. LPS causes fever and endotoxemia by releasing cytokines such as IL-6, finally damaging multiple organs. In addition, it has previously been confirmed that LPS exposure during pregnancy induced hypertension in adult offspring rats [5]. Resistance vessels play crucial roles in regulating blood pressure [6]. Particularly, angiotensin II (AngII), as the strongest vasoconstrictor, predominantly modulates blood pressure. By establishing a hypertensive rat model induced using LPS exposure during pregnancy, we herein assessed the influence of resistance mesenteric vascular function on the development of hypertension in offspring rats.

MATERIALS AND METHODS

Experimental animals and materials

Sprague-Dawley pregnant rats weighing (252.9 ± 15) g were provided by the Animal Experimental Center of our hospital. The animals were housed in accordance with laboratory rat feeding standards and kept at the temperature of (25 ± 1)°C. The offspring rats were weaned at 4 weeks of age and separated by gender in individual cages.

Phenylephrine (PHE), AngII, acetylcholine and losartan were purchased from Sigma (USA). AngII type 1 receptor (AT1R) antibody was bought from Santa Cruz Biotechnology (USA). Goat anti-rabbit fluorescent secondary antibody was obtained from LI-COR Biosciences (USA).

Establishment of LPS stimulation model and grouping

Twenty-four pregnant rats were randomly divided into a control group and an LPS stimulation group which were fed normally, with free access to food and water. The two groups were intraperitoneally injected with 0.5 mL of normal saline and LPS (0.79 mg/kg) on the 8th, 10th and 12th days of pregnancy respectively. The offspring rats were weaned at 4 weeks of age, separated by gender in individual cages and fed normal feed. At 8 weeks of age, 12 male pups were randomly selected from each group for subsequent experiments.

Blood pressure measurement

The blood pressures of offspring rats at 9, 11, 13 and 15 weeks of age were measured noninvasively at the tail using BP-2010 Series (Softron Biotechnology Ltd., China). Before measurement, the two groups were subjected to three adaptive trainings. Blood pressure was measured at 8:00-12:00 am. In detail, the progeny rats were preheated for 5 min at 38° C before measurement, and then placed in a fixing apparatus, with their tails exposed. In the rats' resting state, the tails were sheathed with the balloon and pressure sensor of BP-2010 Series. When the pressure signal became a regular sinusoidal waveform, the systolic blood pressure was recorded. Each rat was tested once every 3 min, and the data of 5 continuous measurements were averaged.

Preparation of isolated mesenteric arterioles

Sixteen 15-week-old offspring rats weighing 250-280 g were taken from each group and anesthetized with 2.5% sodium pentobarbital. Subsequently, the abdominal cavity was opened, and the entire intestinal segment and mesenteric tissue were quickly collected and immediately placed in 4° C normal saline. Then the fat and connective tissue around mesenteric vessels were carefully remove under a microscope while avoiding endothelial damage. A 2 mm-long tertiary vessel of the superior mesenteric artery was cut off, and suspended between two parallel steel wires in a bath chamber containing 5 mL of 37° C nutrient solution which was continuously bubbled with a mixture of 95% O2 and 5% CO2. Model 620 M multichannel wire myograph (Danish Myo Technology, Denmark) was used and connected to PowerLab/8SP data acquisition system (ADInstruments, Australia) through a tension transducer. The vascular tension was optimized, and the mixture was equilibrated at 37° C for 30 min during which the nutrient solution was refreshed every 10 min.

Detection of AngII-mediated contractile responses of mesenteric vessels

When the arterial ring equilibration became stable, 50 mmol PHE was added to contract blood vessels. When the plateau was reached, 50 mmol acetylcholine was added to detect vasodilatability. The endothelium was considered intact if the vasodilatation exceeded 80%, and damaged if not. After 30 min of equilibration, the contractile responses of isolated mesenteric arterioles to 10^{-10}~10^{-5} mol/L AngII were recorded. After another 30 min of equilibration, 50 mmol losartan was added for 30 min of incubation. Finally, the contractile responses were recorded again.

Detection of AT1R expression by Western blotting

Total protein was extracted from the mesenteric artery tissue through grinding in liquid nitrogen, and the concentration was measured by the Coomassie brilliant blue method. Protein samples were resolved by SDS-PAGE and then electronically transferred onto a nitrocellulose membrane. Afterwards, the membrane was blocked for 1 h, incubated with AT1R antibody (1:400 diluted) and GAPDH antibody (1:500 diluted) overnight at 4°C respectively, rewarmed to room temperature for 30 min, washed with TBST three times (10 min each time), incubated with goat anti-rabbit fluorescent secondary antibody (1:150000 diluted) for 1 h, washed again with TBST three times (10 min each time), scanned with a fluorescence scanner and analyzed by Quantity One software.

Statistical analysis

All data were analyzed by SPSS 16.0 software and expressed as mean ± standard deviation ($x \pm s$). Intergroup comparisons were performed by the independent sample t test. $P<0.05$ was considered statistically significant.

RESULTS

General information of offspring rats

Offspring rats in the LPS stimulation group were significantly heavier than those in the control group at 15 weeks of age ($P<0.05$). They had similar levels of blood glucose, creatinine and plasma urea nitrogen ($P>0.05$) (Table 1).

Table 1. General information of offspring rats

	Control group	LPS stimulation group	t	P
Body weight at 15 W (g)	257.46±6.89	275.77±7.21	6.360	<0.001
Blood glucose (mmol/L)	7.51±0.69	7.59±0.70	0.282	0.781
Creatinine (μmol/L)	32.12±2.71	32.45±2.76	0.296	0.770
Plasma urea nitrogen (mmol/L)	5.71±0.72	5.78±0.69	0.243	0.810

Systolic blood pressures of offspring rats

The systolic blood pressures at 9, 11, 13 and 15 W were (114 ± 11) mmHg, (125 ± 12) mmHg, (132 ± 11) mmHg and (139 ± 11) mmHg respectively in LPS group, which were (101 ± 12) mmHg, (112 ± 11) mmHg, (118 ± 12) mmHg and (121 ± 11) mmHg respectively in control group. The systolic blood pressure of the LPS stimulation group was significantly higher than that of the control group at 9, 11, 13 and 15 W ($P<0.05$) (Figure 1).

AngII-induced contractile response of the mesenteric artery in offspring rats

The maximum contractile response of the mesenteric artery induced by AngII in the LPS group exceeded that of the control group [(8.49 ± 0.32) vs. (3.47 ± 0.29) mN, $P<0.05$].When the concentrations of AngII were 10^{-7}, 10^{-6} and 10^{-5} mmol/L, the contractile responses of the mesenteric artery in the LPS group all exceeded those of the control group [(5.99 ± 0.48) vs. (2.21 ± 0.19), (7.10 ± 0.11) vs. (3.02 ± 0.43) and (8.51 ± 0.39) vs. (3.49 ± 0.29) mN respectively, $P<0.01$].After pre-incubation with losartan for 30 min, the contractile response to AngII of each group was significantly inhibited, without a significant difference ($P>0.05$) (Figure 2).

AT1R protein expressions in the mesenteric artery of offspring rats

The relative protein expression of AT1R in the mesenteric arteries of offspring rats was 1.51 ± 0.10 in LPS group and 1.01 ± 0.09 in control group. The protein expression of AT1R in the mesenteric arteries of offspring rats was significantly higher in LPS group than that in control group ($P<0.05$) (Figure 3).

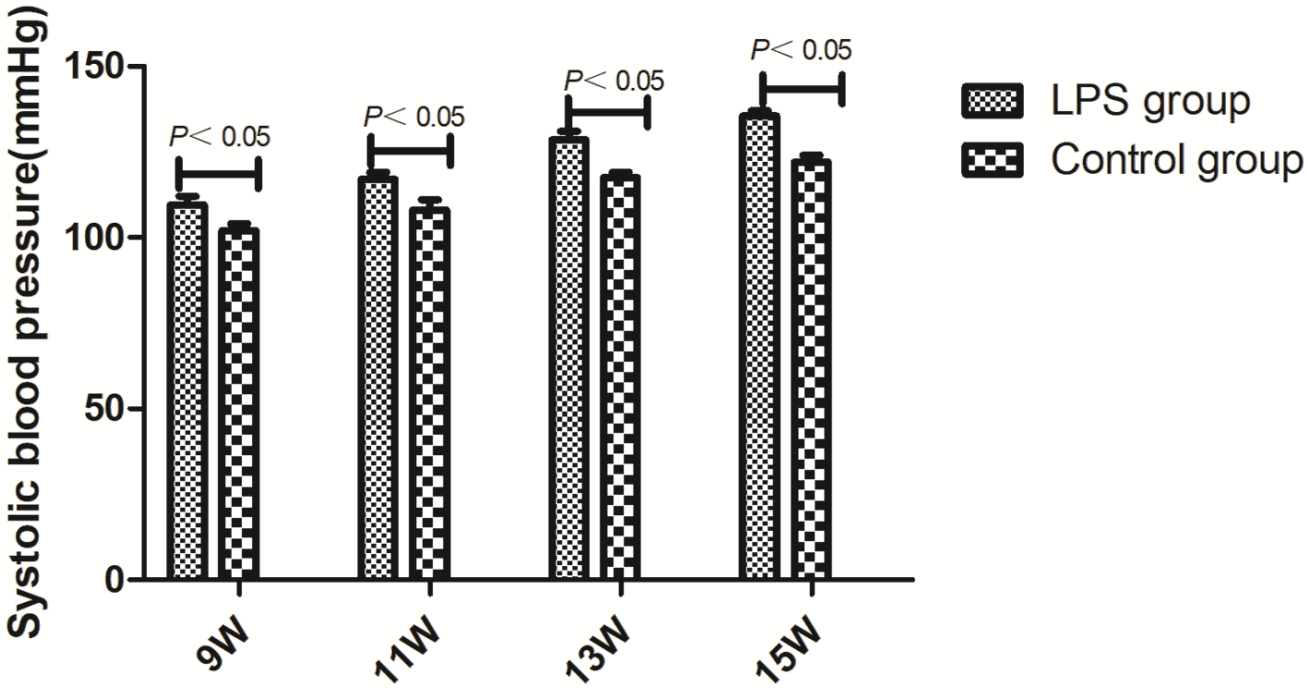

Figure 1. Systolic blood pressures of offspring rats (n=12).

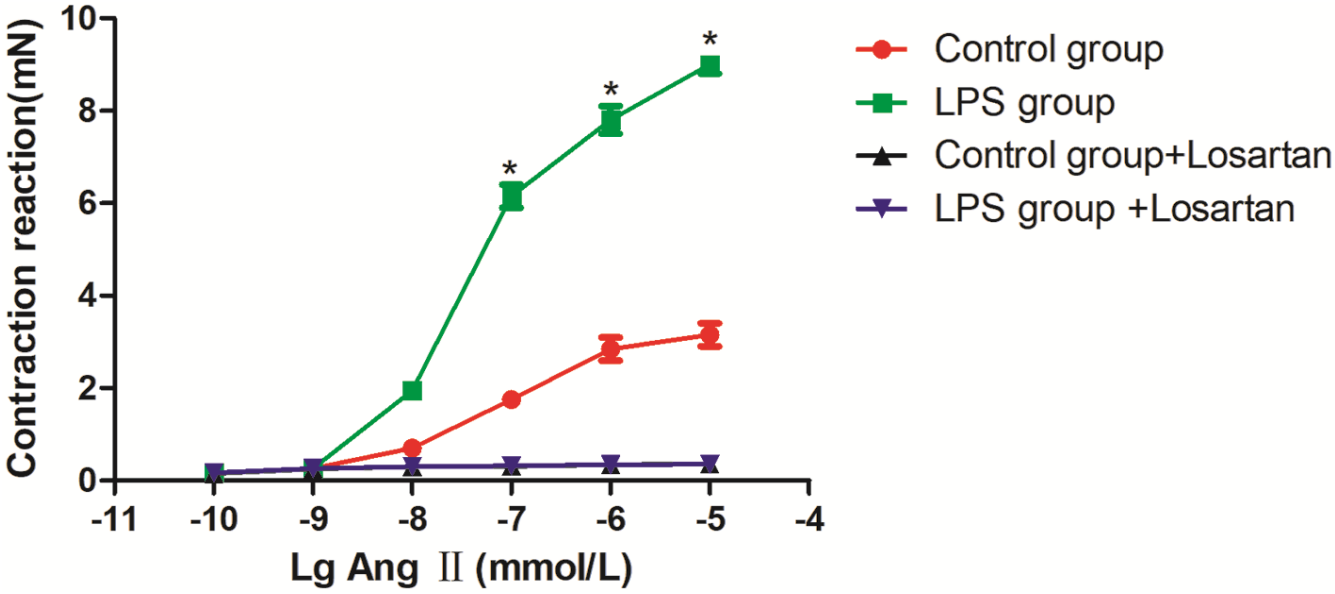

Figure 2. AngII-induced contractile response of the mesenteric artery in offspring rats (n=6).

AT1R protein expressions in the mesenteric artery of offspring rats

The relative protein expression of AT1R in the mesenteric arteries of offspring rats was 1.51 ± 0.10 in LPS group and 1.01 ± 0.09 in control group. The protein expression of AT1R in the mesenteric arteries of offspring rats was significantly higher in LPS group than that in control group (P<0.05) (Figure 3).

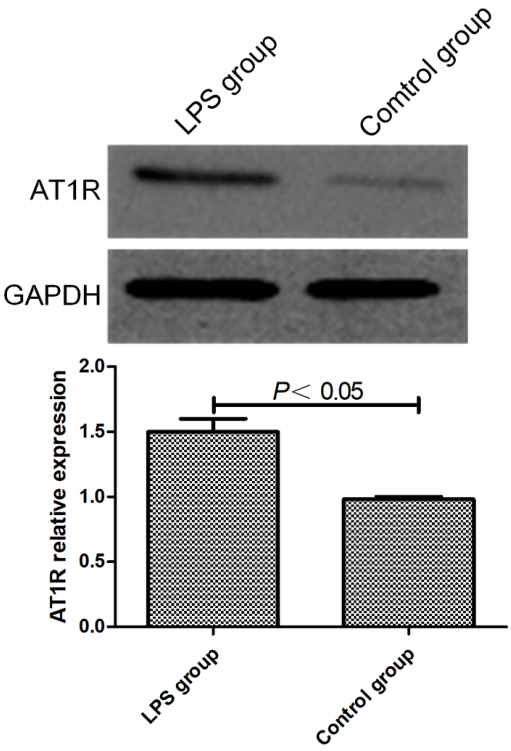

Figure 3. AT1R protein expressions in the mesenteric artery of offspring rats (n=6).

DISCUSSION

The incidence rate of cardiovascular diseases is increasing year by year. As one of the most common cardiovascular diseases, hypertension is one of the most important risk factors for cardiovascular, cerebrovascular and kidney diseases. According to statistical analysis, there have been 266 million hypertensive patients in China currently, as well as more than 10 million new cases of hypertension every year. At present, hypertension is characterized by a high prevalence rate, and low awareness rate, treatment rate and control rate. Despite primary and secondary prevention widely adopted in China and beyond, the prevalence rate of hypertension does not decline but rises, which has become a major public health and medical science issue in China. In previous studies, it was found that the occurrence of hypertension is related to excessive mental stress, high-salt diet and genetics. However, the increasing incidence year by year under widespread primary and secondary prevention around the world indicates the existence of other important pathogenic factors. The possible reason is that previous research and prevention mainly focused on adult individuals. Therefore, it is necessary to further explore new pathogenesis of hypertension, which is of great significance for improving the prevention, control and treatment of hypertension. According to the report of Mazumder in 2010, in a retrospective study on children born to pregnant women during the 1918 influenza pandemic in the United States, it was found that the risk of cardiovascular disease was higher in children born to pregnant women suffering from influenza that year than that in children born to pregnant women not exposed to influenza. Francavilla R et al. observed that periodontal inflammation in pregnant women was closely related to the premature delivery and low birth weight of infants, and they also noted that the incidence rates of hypertension and obesity in premature and low-birth-weight infants in adulthood were also significantly higher than those in normal infants. The above results strongly demonstrate that inflammatory stimulation during pregnancy may be associated with the cardiovascular disease in the offspring.

Fetal nutritional status has significant long-term effects on cardiovascular diseases in adults [4]. Poor stimulation in the uterus causes adaptive changes in embryo, leading to serious or irreversible lifelong damages to the structures or functions of the heart, brain, kidney, blood vessels, pancreas and other organs [7]. During pregnancy, LPS stimulation can cause hypertension in offspring rats by decreasing the number of glomeruli and activating the renin-Ang system [4]. However, peripheral vascular resistance also plays an important role in the progression of hypertension [8-10]. Particularly, peripheral arterioles, such as the mesenteric artery, markedly affects the blood pressure balance [11]. Until now, the influence of LPS stimulation during pregnancy on the peripheral vascular resistance of offspring rats has never been assessed.

AngII is a key substance in the renin-Ang system and the most active vasoconstrictor currently known [12]. As a seven-transmembrane, G protein-coupled receptor, AT1R is widely distributed in mammalian brain, adrenal gland, heart, blood vessels, kidney, liver as well as other tissues and organs [13]. AT1R mediates all the known physiological activities of AngII in cardiovascular and renal tissues, including arterial blood pressure, electrolyte/water balance and regulation of renal function. Upon hypertension, AngII increases the expression of AT1R in endothelial and smooth muscle cells, augments the response to vasoconstrictors, causes contraction of small arteriolar smooth muscles and enhances the peripheral circulation resistance [14]. Under normal conditions, the vasoconstriction and relaxation responses of blood vessels are maintained balanced. If the contractile response is enhanced or the diastolic response is weakened, the tension of the blood vessels becomes unbalanced, causing hypertension [15].

In this study, the blood pressure of progeny rats after LPS stimulated began to increase at 9 weeks of age, which significantly exceeded that of the control group 6 weeks later. Besides, the contractile responses of the mesenteric artery to AngII increased at 15 weeks of age. Accordingly, the enhancement of contractile response may be mainly

responsible for the increase of vascular resistance, finally promoting the onset of hypertension. Moreover, Western blotting showed that the relative protein expression of AT1R in the mesenteric arteries of offspring rats was 1.51 ± 0.10 in LPS group and 1.01 ± 0.09 in control group. The protein expression of AT1R in the mesenteric arteries of offspring rats was significantly higher in LPS group than that in control group ($P<0.05$). AT1R protein expression in the mesenteric artery of offspring rats of the LPS stimulation group was increased, which also confirmed the enhancement of AT1R function. Collectively, the balance between mesenteric vasoconstriction and relaxation in offspring rats was broken after LPS stimulation during pregnancy, thereby augmenting AngII-mediated contractile response, vascular resistance and blood pressure.

In conclusion, exposure to LPS during pregnancy leads to abnormal AngII-mediated vasoconstriction, and raises the vascular resistance and blood pressure in offspring rats.The findings verify the relationship between poor stimulation during pregnancy and offspring hypertension, and also provide valuable evidence for preventing and treating hypertension in clinical practice.

Acknowledgments

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