High-Salt Diet Enhances Vascular Reactivity in Pregnant Rats With Normal and Reduced Uterine Perfusion Pressure

Laura A. Barron, Jena. B. Giardina, Joey P. Granger, Raouf A. Khalil

Abstract—High-salt (HS) diet is often associated with increased vascular resistance and arterial pressure; however, the effects of HS intake on the vascular control mechanisms of arterial pressure during pregnancy are unclear. We investigated whether a HS diet during pregnancy is associated with increases in vascular reactivity. Active stress was measured in aortic strips of virgin and normal pregnant Sprague-Dawley rats and a hypertensive pregnant rat model produced by reduction in uterine perfusion pressure (RUPP), fed either normal-sodium (NS, 1%) or HS diet (8%) for 7 days. In endothelium-intact strips, phenylephrine (Phe) caused a concentration-dependent contraction that was greater in RUPP rats than in normal pregnant or virgin rats and was significantly enhanced in pregnant/HS and RUPP/HS rats compared with pregnant/NS and RUPP/NS rats, respectively. Removal of the endothelium enhanced the Phe-induced stress slightly in virgin rats and significantly in pregnant/NS but not in pregnant/HS, RUPP/NS, or RUPP/HS. In endothelium-intact strips, acetylcholine (ACh) caused a concentration-dependent relaxation that was reduced in RUPP/NS (max, 31%) compared with pregnant/NS rats (max, 75%). ACh relaxation was further reduced in pregnant/HS rats compared with RUPP/NS rats and in RUPP/HS rats compared with RUPP/NS rats. Pretreatment of endothelium-intact strips with N\(^5\)-nitro-L-arginine methyl ester (L-NAME, 10\(^{-4}\) mol/L), to inhibit NO synthase, or with 1H-[1,2,4]oxadiazolo[4,3-b]quinoxalin-1-one (ODQ, 10\(^{-6}\) mol/L), to inhibit cGMP production in smooth muscle, inhibited ACh-induced relaxation and enhanced Phe-induced contraction in pregnant/NS rats but not in pregnant/HS, RUPP/NS, or RUPP/HS rats. Basal and ACh-induced nitrite/nitrate production from aortic strips showed significant reduction in pregnant/HS rats compared with pregnant/NS rats but not in RUPP/HS rats compared with RUPP/NS rats. Sodium nitroprusside, an exogenous NO donor, caused relaxation of Phe contraction that was similar in virgin or pregnant rats on an NS or HS diet but was significantly reduced in RUPP/HS rats (ED\(_{50}\) 6\(\times\)10\(^{-8}\) mol/L) compared with RUPP/NS rats (ED\(_{50}\) 6\(\times\)10\(^{-9}\) mol/L). Thus, a HS diet in normal pregnant and RUPP rats is associated with increases in vascular reactivity. The enhanced vascular reactivity with the HS diet is possibly related to abnormalities in NO synthesis/release from the endothelium in normal pregnant rats and an additional decrease in the sensitivity of the smooth muscle to relaxation by NO in pregnant rats with reduced uterine perfusion pressure. (Hypertension. 2001; 38[part 2]:730-735.)

Key Words: arterial pressure ■ endothelium ■ nitric oxide ■ muscle, smooth, vascular ■ contraction

High-salt (HS) diet has been implicated in the pathogenesis of hypertension, particularly in salt-sensitive individuals, and salt moderation is often recommended to protect against excessive increases in vascular resistance and arterial pressure.\(^1,2\) However, the interrelationship between sodium intake, vascular resistance, and blood pressure regulation during pregnancy is less clear. During normal pregnancy, reductions in systemic vascular resistance, arterial pressure, and vascular reactivity to circulating vasoconstrictors are often observed.\(^3,4,6-8\) The hemodynamic and vascular changes during normal pregnancy have been attributed, in part, to increased NO synthesis by various cells, including vascular endothelial cells.\(^7,9\) In 5% to 7% of pregnancies, women develop a condition called preeclampsia, which is characterized by increased intravascular coagulation, proteinuria, increased systemic vascular resistance, and pregnancy-induced hypertension.\(^10,11\) Although pregnancy-induced hypertension is a major cause of maternal and fetal mortality, the exact mechanism of this disorder has not yet been clearly identified. Studies in pregnant animal models have suggested that a reduction in the utero-placental perfusion pressure and the ensuing placental ischemia during late pregnancy are associated with placental release of cytokines, which eventually leads to increased systemic vascular resistance and pregnancy-induced hypertension.\(^12-14\) We have recently used a pregnant rat model with reduced uterine perfusion pressure (RUPP), produced by clipping the lower abdominal aorta and the main uterine branches of both the right and left ovarian...
arteries, and found that the vascular reactivity is significantly enhanced in RUPP rats compared with normal pregnant rats.

Although HS diet has been shown to be associated with significant elevations of arterial pressure in pregnant animal models, particularly those with reduced uterine perfusion pressure, the vascular mechanisms involved are unclear. The purpose of the present study was to determine (1) whether the vascular reactivity to the α-adrenergic agonist phenylephrine is enhanced in normal pregnant or RUPP rats during a HS diet; (2) whether endothelin-dependent vascular relaxation is reduced in pregnant and RUPP rats during a HS diet; and (3) whether the changes in vascular relaxation and vascular reactivity associated with a HS diet during pregnancy involve alterations in the endothelin-dependent NO-cGMP pathway. The last question was an aim of the present study because NO is a major endothelium-derived relaxing factor and because normal pregnancy is associated with significant increase in NO production.

**Methods**

**Animals**

Virgin and time-pregnant (day 12) Sprague-Dawley rats (12 weeks) were housed individually and maintained on ad libitum standard rat chow and tap water on a 12-hour/12-hour light/dark cycle. The rats were divided into 6 groups with 12 rats each: virgin on normal-salt (NS) diet, virgin on HS diet, normal pregnant/NS, normal pregnant/HS, RUPP/NS, and RUPP/HS. The NS rats were fed a diet containing 1% sodium chloride. The HS rats were fed a diet containing 8% sodium chloride. The rats were kept on their respective diets for 7 days. The water intake was greater in the HS rats compared with the NS groups, as reflected by a 4- to 5-fold increase in urine volume.

All procedures were performed in accordance with the guidelines of the Animal Care and Use Committee at the University of Mississippi Medical Center.

**Protocol for RUPP**

On day 14 of pregnancy, pregnant rats that were to be in the RUPP group were anesthetized with isoflurane. The abdominal cavity was opened by a midline incision, the lower abdominal aorta was exposed, and a silver clip (0.229 mm ID) was placed around the aorta above the iliac bifurcation. This procedure reduces uterine perfusion by 40%. Because compensation of blood flow to the placenta occurs through an adaptive increase in ovarian blood flow, a silver clip (0.1 mm ID) was also placed on the main uterine branches of both the right and left ovarian arteries. Control pregnant and virgin rats were sham-operated. Using this protocol, the RUPP rats showed features similar to those observed in preeclampsia in women, including proteinuria, impaired renal function, and intrauterine growth retardation as indicated by decreased litter size and pup weight. Although the maternal body weight and fetal weight were reduced in RUPP rats compared with normal pregnant rats, the maternal and fetal weight were not significantly different between the HS and NS groups. RUPP rats in which the clipping procedure resulted in maternal death or total reabsorption of the fetuses were excluded from the study.

**Measurement of Mean Arterial Pressure**

All rats were anesthetized with isoflurane and underwent a surgical procedure to implant a PE-50 arterial catheter in the carotid artery for measurement of mean arterial pressure. The catheter was filled with heparin and exteriorized at the back of the neck. Rats were housed individually in metabolic cages and allowed to recover for 48 hours. On the day of the experiment (typically day 19 of gestation or the equivalent in virgin rats), each rat was placed in a Plexiglass restrainer. The carotid arterial catheter was connected to a Statham pressure transducer, and the arterial pressure in conscious rats was recorded on a Grass polygraph (Model 7D).

**Tissue Preparation**

On the day of the experiment, the rats were anesthetized by inhalation of isoflurane. The thoracic aorta was excised, placed in oxygenated Krebs solution, and cleaned of connective tissue. The aorta was cut transversely into 3-mm-wide strips. To get endothelium-denuded aortic strips, the endothelium was removed by gently rubbing the vessel interior with wet filter paper.

**Isometric Contraction**

One end of the aortic strip was attached to a glass hook using a thread loop, and the other end was connected to a Grass force transducer (FT03). Aortic strips were stretched to Lmax (1.5× the unloaded initial length, L). The strips were allowed to equilibrate for 1 hour in a water-jacketed, temperature-controlled tissue bath filled with 50 mL Krebs solution continuously bubbled with 95% O2/5% CO2 at 37°C. The changes in isometric contraction were recorded on a Grass polygraph (Model 7D).

Two control phenylephrine (Phe, 10^{-5} mol/L) contractions followed by rinsing with Krebs solution 3×10 minutes were first performed. Increasing concentrations of Phe were applied, and concentration-contraction curves were constructed. In other tissues, a contraction to submaximal concentration of Phe (3×10^{-2} mol/L) was elicited. Acetylcholine (ACh) or sodium nitroprusside was added, and the vascular relaxation was measured. In other experiments, the tissues were pretreated for 30 minutes with N^o-nitro-l-arginine methyl ester (L-NAME, 10^{-4} mol/L), to inhibit NO synthase, or 1H-[1,2,4]oxadiazolo[4,3-b]quinazolin-1-one (ODQ, 10^{-6} mol/L), to inhibit cGMP production in smooth muscle, and the effects on the Phe-induced contraction and on the ACh-induced relaxation of Phe contraction were observed.

**Nitrite/Nitrate Production**

Endothelium-intact aortic strips were placed in test tubes containing 2 mL Krebs aerated with 95% O2/5% CO2 at 37°C, and the solution was changed every 30 minutes for 1 hour. Samples for basal accumulation of nitrite formed from released NO were first taken. The Krebs solution was replaced, and the tissue strips were stimulated with ACh for 5 minutes. The strips were rapidly removed, dabbed dry with tissue paper, and weighed. The incubation solutions were assayed for the stable end product of NO, NO2^{-}. Briefly, samples of incubation solution (50 μL, in triplicate) were mixed in a 96-well microtiter plate with 100 μL of the Griess reagent. The chromophore generated by the reaction with nitrite was detected spectrophotometrically (550 nm) using a microtiter plate reader (BioTek). The concentration of nitrate was calculated using a calibration curve with known concentrations of NaNO2.

**Solutions and Chemicals**

Normal Krebs contained the following (in mmol/L): NaCl 120, KCl 5.9, NaHCO3 25, NaHPO4 1.2, dextrose 11.5, MgCl2 1.2, and CaCl2 2.5, at pH to 7.4. L-phenylephrine, acetylcholine, sodium nitroprusside, and L-NAME (Sigma) were prepared in distilled water. ODQ (Calbiochem) was dissolved in dimethyl sulfoxide (final concentration, <0.1). All other chemicals were of reagent grade or better.

**Statistical Analysis**

The developed force was corrected for the cross sectional area of each individual strip and expressed as active stress (N/m) using the equation: stress=[force/cross sectional area], where cross sectional area=[wet weight/(tissue density×length of the strip)] and tissue density=1.055 g/cm³. Data were analyzed and expressed as mean±SEM. Data were compared using ANOVA with multiple classification criteria (rat type [NS versus HS, normal pregnant
versus RUPP), condition of endothelium (intact versus denuded), and treatment (untreated versus pretreated with L-NAME or ODQ) followed by Bonferroni’s post test to compare selected groups or Dunnett’s post test to compare all groups to the pregnant/NS group. Differences were considered statistically significant if $P < 0.05$.

**Results**

On the day of the experiment, the mean arterial pressure in virgin/NS rats (108±8 mm Hg) was not significantly different from that of virgin/HS rats (112±10 mm Hg). The arterial pressure was significantly greater in pregnant/HS rats (119±3 mm Hg) compared with pregnant/NS rats (102±3 mm Hg) and in RUPP/HS rats (140±8 mm Hg) compared with RUPP/NS rats (119±6 mm Hg). The arterial pressure in RUPP rats on an NS or HS diet was significantly greater than that in normal pregnant rats on an NS or HS diet, respectively.

In aortic strips of virgin, normal pregnant, and RUPP rats, Phe caused concentration-dependent increases in active stress (Figure 1). In endothelium-intact strips, the Phe concentration-active stress curve was slightly greater in virgin/HS rats than in virgin/NS rats (Figure 1A) but significantly greater in pregnant/HS rats than in pregnant/NS rats (Figure 1B) and in RUPP/HS rats than in RUPP/NS rats (Figure 1C). Removal of the endothelium enhanced the Phe-induced stress slightly in virgin rats (Figure 1A) and significantly in pregnant/NS rats (Figure 1B) but not in pregnant/HS (Figure 1B), RUPP/NS, or RUPP/HS rats (Figure 1C). When the Phe response was presented as percentage of the maximum Phe contraction, Phe was slightly more potent in virgin/HS rats than in virgin/NS rats (Figure 1D) but significantly more potent in pregnant/HS rats than in pregnant/NS rats (Figure 1E) and in RUPP/HS rats than in RUPP/NS rats (Figure 1F). Phe was more potent in causing contraction in endothelium-denuded than endothelium-intact aortic strips of virgin (Figure 1D) and pregnant/NS rats (Figure 1E) but not in pregnant/HS (Figure 1E), RUPP/NS, or RUPP/HS rats (Figure 1F).

In endothelium-intact strips, pretreatment with L-NAME ($10^{-4}$ mol/L) for 30 minutes to inhibit NO synthase significantly enhanced the Phe-induced stress in virgin/NS and virgin/HS rats (Figure 2A). L-NAME enhanced the Phe-induced stress in pregnant/NS rats but not in pregnant/HS rats (Figure 2B). In contrast, L-NAME did not enhance the Phe-induced contraction in RUPP/NS or RUPP/HS rats (Figure 2C).

Similarly, in endothelium-intact strips, pretreatment with ODQ ($10^{-6}$ mol/L) for 30 minutes to inhibit cGMP produc-
tion in smooth muscle significantly enhanced the Phe-induced stress in virgin/NS and virgin/HS rats (Figure 2A). ODQ enhanced the Phe-induced stress in pregnant/NS rats but not in pregnant/HS (Figure 2B), RUPP/NS, or RUPP/HS rats (Figure 2C).

In endothelium-intact aortic strips of virgin, normal pregnant, and RUPP rats, ACh caused concentration-dependent relaxation of Phe (3\times10^{-7} \text{ mol/L}) contraction (Figure 3A). The ACh-induced relaxation was significantly greater in normal pregnant rats than in RUPP rats. Because the aortic strips of RUPP rats showed greater vascular reactivity than normal pregnant rats, control experiments were performed on strips of RUPP rats: the initial Phe concentration was lowered to 10^{-7} \text{ mol/L} to produce a submaximal contraction that is roughly equal in magnitude to the contraction observed in strips of pregnant rats precontracted with 3\times10^{-7} \text{ mol/L} Phe. The ED_{50} of ACh in aortic strips of RUPP rats precontracted with 10^{-7} \text{ mol/L} Phe (1.1\pm0.1\times10^{-6} \text{ mol/L}) was not significantly different from that in strips precontracted with 3\times10^{-7} \text{ mol/L} Phe (1.2\pm0.1\times10^{-6} \text{ mol/L}).

The ACh relaxation was not significantly different between virgin/HS and virgin/NS rats but was significantly reduced in pregnant/HS rats compared with pregnant/NS rats and in RUPP/HS rats compared with RUPP/NS rats. Pretreatment of endothelium-intact strips with L-NAME (10^{-4} \text{ mol/L}) or ODQ (10^{-6} \text{ mol/L}) significantly inhibited the ACh-induced relaxation of Phe contraction in virgin/NS and virgin/HS rats. L-NAME and ODQ inhibited ACh relaxation significantly in pregnant/NS rats, but only slightly in pregnant/HS rats. L-NAME and ODQ did not significantly affect ACh relaxation in RUPP/NS or RUPP/HS rats. Removal of the endothelium completely inhibited the ACh-induced relaxation of Phe contraction in virgin, normal pregnant, and RUPP rats on an NS or HS diet (data not shown).

In endothelium-intact strips, the basal nitrite/nitrate production was significantly greater in virgin/HS rats than in virgin/NS rats, but the ACh-induced nitrite/nitrate production was not significantly different (Figure 3B). The basal and ACh-induced nitrite/nitrate showed significant reduction in pregnant/HS rats compared with pregnant/NS rats but not in RUPP/HS rats compared with RUPP/NS rats (Figure 3B).

In endothelium-denuded aortic strips sodium nitroprusside, an exogenous NO donor and a standard guanylate cyclase activator, caused concentration-dependent relaxation of Phe contraction (Figure 4). Sodium nitroprusside-induced relaxation of Phe contraction was not significantly different between virgin/HS and virgin/NS rats (Figure 4A) or between pregnant/HS and pregnant/NS rats (Figure 4B) but was significantly reduced in RUPP/HS rats compared with RUPP/NS rats (Figure 4C).

**Discussion**

The main findings of this study are that (1) arterial pressure is significantly greater in normal pregnant and RUPP rats on...
a HS diet than those on an NS diet; (2) vascular reactivity is greater in pregnant and RUPP rats on a HS diet compared with those on an NS diet; (3) endothelium-dependent vascular relaxation is reduced in pregnant/HS rats compared with pregnant/NS rats and further reduced in RUPP/HS rats compared with RUPP/NS rats.

We found that a HS diet in Sprague-Dawley virgin rats caused minimal change in arterial pressure. This is consistent with reports that feeding Dahl salt-resistant rats a HS diet is associated with modest elevation in blood pressure. The arterial pressure was significantly greater in RUPP rats than in normal pregnant rats, which is consistent with previous reports that the arterial pressure is enhanced in animal models of hypertension during late pregnancy. The observations that the vascular reactivity to Phe was greater in RUPP rats than in normal pregnant rats, that removal of the endothelium enhanced Phe contraction in normal pregnant rats but not in RUPP rats, and that ACh-induced relaxation was reduced in RUPP rats compared with normal pregnant rats are consistent with our previous findings and suggest that an endothelium-dependent relaxation pathway is intact in normal pregnant rats but is impaired in RUPP rats.15,16

The arterial pressure was significantly increased in pregnant/HS rats compared with pregnant/NS rats and in RUPP/HS rats compared with RUPP/NS rats. This is consistent with reports that a HS diet is associated with significant elevations of arterial pressure in normal pregnant rabbits17 and in pregnant sheep with RUPP.18 In search for the possible vascular mechanisms involved in the observed elevated arterial pressure in pregnant and RUPP rats on a HS diet, we found that the vascular reactivity to Phe is enhanced in pregnant/HS and RUPP/HS rats compared with pregnant/NS and RUPP/NS rats, respectively. Removal of the endothelium enhanced the Phe contraction significantly in pregnant/NS rats but not in pregnant/HS rats and had minimal effect in RUPP/NS and RUPP/HS rats. Also, the ACh-induced relaxation was reduced significantly in pregnant/HS rats compared with pregnant/NS rats and in RUPP/HS rats compared with RUPP/NS rats. These data suggest that an endothelium-dependent relaxation pathway is impaired in pregnant rats on a HS diet and further impaired in RUPP rats on a HS diet.

The vascular endothelium releases several vasodilator substances including NO. The reduced ACh-induced relaxation in pregnant/HS and RUPP/HS rats could be due to a decrease in the synthesis/release of NO from endothelial cells or to a decrease in the sensitivity of vascular smooth muscle to relaxation by NO. We found that pretreatment of the vascular strips with L-NAME, which blocks NO synthesis, inhibited ACh-induced relaxation and enhanced the vascular reactivity to Phe significantly in pregnant/NS rats but not in pregnant/HS rats and had minimal effects in RUPP rats on an NS or HS diet. These results suggest that NO synthesis by endothelial cells is intact in pregnant/NS rats but is impaired with a HS diet in normal pregnant and RUPP rats. This is supported by the observation that the basal and ACh-induced nitrite/nitrate production were reduced in pregnant/HS rats compared with pregnant/NS rats. The NO synthesis/release appeared to be maximally inhibited in RUPP/NS rats, because no additional decrease in the nitrite/nitrate production was observed in RUPP/HS rats compared with RUPP/NS rats.

The NO produced by endothelial cells promotes vascular relaxation by activating guanylate cyclase and increasing cGMP in vascular smooth muscle.26 We found that ODQ, which inhibits guanylate cyclase and decreases cGMP in smooth muscle,21 inhibited the ACh-induced relaxation and enhanced the vascular reactivity to Phe significantly in endothelium-intact strips of pregnant/NS rats but not in pregnant/HS rats and had minimal effects in RUPP rats on an NS or HS diet. These results further support that NO production/release by endothelial cells, and thereby the activity of the NO-cGMP relaxation pathway in vascular smooth muscle, is reduced in pregnant/HS rats compared with pregnant/NS rats. The NO-cGMP pathway appeared to be maximally inhibited in RUPP/NS rats, because ODQ did not cause further reduction of ACh-relaxation or enhancement of Phe contraction in RUPP/NS rats compared with RUPP/HS rats.

The question arises as to how the ACh-induced relaxation was further inhibited in RUPP/HS rats compared with RUPP/NS rats in the absence of significant difference in nitrite/nitrate production. This may be related to the sensitivity of vascular smooth muscle to relaxation by NO. The observation that relaxation of endothelium-denuded vascular strips by sodium nitroprusside was not significantly different between pregnant/HS and pregnant/NS rats but was significantly reduced in RUPP/HS rats compared with RUPP/NS rats suggests that the sensitivity of vascular smooth muscle to relaxation by NO is impaired in RUPP rats on a HS diet. The cause of the decreased vascular relaxation by NO in RUPP rats on a HS diet is unclear at the present time but could be related to abnormalities in the amount or activity of the guanylate cyclase or the cGMP-dependent protein kinase (protein kinase G) system or to structural changes within the arterial wall and should represent important areas for future investigations.

We should emphasize that the vascular endothelium releases other vasodilator substances in addition to NO, such as endothelium-derived hyperpolarizing factor and prostacyclin.29 This may explain why in the aortic strips of RUPP rats some relaxation to ACh was still observed and was not inhibited by L-NAME or ODQ. Whether the production of vasodilators other than NO is impaired in pregnant and RUPP rats on a HS diet remains to be investigated. Also, although the present results provided evidence that the enhanced vascular reactivity in the pregnant and RUPP rats on a HS diet may involve inhibition of an endothelium-dependent relaxation pathway, we cannot rule out the possibility that an increase in the release of contracting factors from the endothelium or an increase in the sensitivity of vascular smooth muscle to endothelium-derived contracting factors also occurs. This is supported by a recent report that long-term inhibition of NO synthesis during mid to late gestation in rats is associated with elevated plasma levels of endothelin-1.30 Thus, a HS diet during normal pregnancy or during reduction of uterine perfusion pressure in late pregnant rats may be associated with additional alterations in the cellular mechanisms of vascular smooth muscle contraction. The enhanced
Phe-induced smooth muscle contraction with a HS diet during pregnancy could be related, in part, to increases in the sensitivity to Phe at the α-adrenergic receptor level. This is supported by the observations that when the Phe response was presented as percentage of maximum Phe contraction, Phe was more potent in pregnant/NS rats than in pregnant/NS rats and in RUPP/HS rats than in RUPP/NS rats. However, the enhanced Phe contraction with a HS diet could also be related to possible changes in the signaling mechanisms downstream from α-adrenergic receptor activation or perhaps changes in the number or size of the smooth muscle cells and should represent important areas for future investigations.

In conclusion, a HS diet in normal pregnant and RUPP rats is associated with increases in vascular reactivity. The enhanced vascular reactivity with a HS diet may be related to abnormalities in NO production/release from the endothelium in normal pregnant rats; and an additional decrease in the sensitivity of the smooth muscle, to relaxation by NO in pregnant rats with reduced uterine perfusion pressure.

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References
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