Systemic hemodynamic and regional blood flow changes in response to chronic reductions in uterine perfusion pressure in pregnant rats

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Sholook MM, Gilbert JS, Sedeeck MH, Huang M, Hester RL, Granger JP. Systemic hemodynamic and regional blood flow changes in response to chronic reductions in uterine perfusion pressure in pregnant rats. Am J Physiol Heart Circ Physiol 293: H2080–H2084, 2007. First published July 20, 2007; doi:10.1152/ajpheart.00667.2007.—Preeclampsia (PE) is associated with increased total peripheral resistance (TPR), reduced cardiac output (CO), and diminished uterine and placental blood flow. We have developed an animal model that employs chronic reductions in uterine perfusion pressure (RUPP) in pregnant rats to generate a “preeclamptic-like” state during late gestation that is characterized by hypertension, proteinuria, and endothelial dysfunction. Although this animal model has many characteristics of human PE, the systemic hemodynamic and regional changes in blood flow that occur in response to chronic RUPP remains unknown. Therefore, we hypothesized that RUPP would decrease uteroplacental blood flow and CO, and increase TPR. Mean arterial pressure (MAP), CO, cardiac index (CI), TPR, and regional blood flow to various tissues were measured using radiolabeled microspheres in the following two groups of conscious rats: normal pregnant rats (NP; n = 8) and RUPP rats (n = 8). MAP was increased (132 ± 4 vs. 99 ± 3 mmHg) in the RUPP rats compared with the NP dams. The hypertension in RUPP rats was associated with increased TPR (2.15 ± 0.02 vs. 0.98 ± 0.08 mmHg·ml⁻¹·min⁻¹) and decreased CI (246 ± 20 vs. 348 ± 19 ml·min⁻¹·kg⁻¹, P < 0.002) when contrasted with NP dams. Furthermore, uterine (0.16 ± 0.03 vs. 0.38 ± 0.09 ml·min⁻¹·g tissue⁻¹) and placental blood flow (0.30 ± 0.08 vs. 0.70 ± 0.10 ml·min⁻¹·g tissue⁻¹) were decreased in RUPP compared with the NP dams. These data demonstrate that the RUPP model of pregnancy-induced hypertension has systemic hemodynamic and regional blood flow alterations that are strikingly similar to those observed in women with PE.

PREECLAMPSIA (ALSO TERMED pregnancy-induced hypertension, PIH) is a significant source of maternal and neonatal morbidity and mortality and historically has affected ~8–10% of all pregnancies (28). Moreover, the incidence of preeclampsia has risen 40% in the last decade (27), and experimental and clinical evidence indicates that offspring of preeclamptic mothers are at increased risk of developing hypertension (5, 6, 31, 32). Despite being one of the leading causes of maternal death and a major contributor of maternal and perinatal morbidity, the mechanisms responsible for pathogenesis of PIH remain nebulous. The uncertainties regarding the mechanisms of PIH are at least partially attributable to the difficulties in developing suitable animal models for mechanistic research of this disease (25). Consequently, it is held by many that more effective strategies for prevention and treatment of PIH will be forthcoming with the recent progress in developing animal models that allow careful investigation of the underlying pathophysiological mechanisms involved in PIH (17).

Normal pregnancy is characterized by marked cardiovascular adaptations that are required to support proper fetal growth and development and to culminate in a successful pregnancy. These adaptations include a dramatic increase in uteroplacental blood flow that also occurs to accommodate the increasing needs of the growing fetus and a large increase in cardiac output (CO) that is concomitant with a decrease in total peripheral resistance (TPR), which allows the maintenance of proper arterial pressure (AP; see Refs. 9, 11, and 22). In contrast, pregnancies affected by PIH are typified by increased TPR and mean arterial pressure (MAP) and decreased CO and uteroplacental blood flow (8, 24, 33).

Experimental induction of chronic uteroplacental ischemia in gravid rats [reduction in uterine perfusion pressure (RUPP) model] is a promising animal model for studying the mechanisms and consequences of PIH (17, 25). Moreover, the RUPP model of PIH in rats is associated with proteinuria, endothelial dysfunction, reduced renal plasma flow, glomerular filtration rate, and nitric oxide production, and increased oxidative stress, many of which are observed in pregnant women presenting with preeclampsia (1, 4, 12, 21). Although RUPP-induced hypertension in the pregnant rat has many of the features of PIH in women, no information is yet available regarding systemic hemodynamics and regional blood flow alterations associated with chronic RUPP.

Therefore, we hypothesized that the RUPP maneuver in pregnant rats would elicit cardiovascular manifestations similar to those observed in preeclamptic women, i.e., decreased CO and uteroplacental blood flow and increased TPR. To that end, we employed our RUPP model of PIH to evaluate CO, AP, and regional blood flow by way of radiolabeled microspheres in pregnant rats at day 18 of gestation.

METHODS

Animals. Studies were performed in timed-pregnant Sprague-Dawley rats (Harlan, Indianapolis, IN) in which day of gestation was

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determined by observation of a vaginal plug. Plug date was considered to be day 0 of gestation. Rats were housed in a temperature-controlled room (23°C) with a 12:12-h light-dark cycle and were given free choice access to water and standard rat chow (Harlan Teklad, Madison, WI). All experimental procedures executed in this study were in accordance with National Institutes of Health guidelines for use and care of animals and were approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center. On day 14 of gestation, rat dams were randomly assigned to either RUPP (n = 8) or normal pregnant (NP; n = 8) control groups.

**RUPP procedure.** The RUPP procedure is a well-established model for simulating preeclampsia in the pregnant rat and has been described in detail previously (18). In brief, all rats undergoing surgical procedures were anesthetized with 2% isoflurane. Sixteen pregnant rats were randomly assigned to either the NP control or the RUPP group. Pregnant rats entering the RUPP group underwent the following clipping procedure at day 14 of gestation. After a midline incision, the lower abdominal aorta was isolated, and a silver clip (0.203 mm ID) was placed around the aorta above the iliac bifurcation. To prevent augmentation of blood flow to the uterus via the ovarian arteries, silver clips (0.100 mm ID) were also placed on branches of both the right and left ovarian arteries that supply the uterus. We have previously shown that this maneuver does not impair ovarian function as determined by circulating concentrations of 17β-estradiol and progesterone (3). NP dams underwent a sham procedure consisting of an abdominal incision, isolation of the abdominal aorta and ovarian arteries, and concluding with closure of the abdominal cavity. When the clipping procedure resulted in total reabsorption of the fetuses, rats were excluded from data analyses.

**Measurement of hemodynamics.** At day 17 gestation, during isoflurane anesthesia as described above, rats in the NP group were surgically instrumented with a left femoral artery catheter (PE-50) for blood pressure monitoring and blood withdrawal of the reference sample. In RUPP rats, because of the presence of the aortic clip, similar catheters were inserted in the left axillary arteries. The rats were allowed to recover from anesthesia for ~10 h. To obtain blood pressure measurements, each rat was placed in a Plexiglas restrainer of suitable size, the arterial catheter was connected to a Statham pressure transducer, and MAP was continuously recorded as described previously (15).

At day 18 of gestation, both groups of rats were prepared for microsphere injection as described previously (19). Briefly, under isoflurane anesthesia, a lubricated catheter of heat-stretched PE-50 tubing was inserted in the right carotid artery and advanced in left ventricle of the heart. Placement was confirmed by both pulse pressure tracing and postpartum presence of the catheter tip within the left ventricle. The rats were allowed to recover from anesthesia for ~3 h. Following the determination of blood pressure, CO and regional hemodynamic measurements were obtained in the following manner. Microspheres radiolabeled with 46Sc (New England Nuclear) with a mean diameter of 15 ± 3 μm were purchased dry and suspended in a 1.3 specific gravity dextrose solution with one drop of 0.05% Tween 80 to prevent clumping of microspheres as previously described (30). The microsphere solution was dispersed by using an ultrasonic bath (Radiograph) for 5 min and then mixed with a vortex shaker for 3 min. Precoiled PE-50 tubing (72 cm long, 0.2 ml filling volume) was filled with the microsphere solution, which was adjusted to a dilution to contain 300,000 microspheres/coil. The coils were sealed with metal plugs at both ends, and radioactivity was determined by using a gamma counter (Nuclear Chicago Series 1185). Each rat was prepared for microsphere injection in the conscious state by interposing the preloaded coil between the left ventricular catheter and a Gilford infusion pump and connecting the femoral catheter to a withdrawal pump of the same type. Microspheres were injected in the left ventricle and flushed with 0.6 ml of saline over a period of 20 s. The withdrawal pump was adjusted to collect a 1-ml blood sample from the femoral artery over a period of 90 s starting at the same time as the microsphere injection.

**CO and regional blood flow measurements.** After the injection had been completed, all rats were euthanized by injection of supersaturated KCl while under isoflurane anesthesia. Individual placenta, uterus, ovaries, heart, kidneys, brain, lungs, liver, stomach, intestine, and skeletal muscles of right hindlimbs were carefully dissected and weighed.

The arterial reference flow was calculated by the following equation:

\[
\text{Weight of the reference blood sample} \times 60 \text{ (s/min)} = \frac{1.05 \text{ (specific gravity of blood)}}{\text{time withdrawal (s)}} \times \text{CO}
\]

Weight of the reference blood sample \(\times 60\) (s/min) = 1.05 (specific gravity of blood) \(\times\) time withdrawal (s) \(\times\) CO was calculated using the following formula:

\[
\text{CO (ml/min)} = \frac{\text{arterial reference flow (ml/min)}}{\text{no. of microspheres injected}} \times \text{CO}
\]

Tissue and organ flow were determined as follows:

\[
\text{Organ blood flow (ml min}^{-1} \text{g}^{-1} \text{tissue} = \frac{(}\text{no. of microspheres in the tissue/no. of injected microspheres}) \times \text{CO}
\]

Cardiac index (CI) was expressed as CO per kilogram of body wt (ml·min\(^{-1}\)·g\(^{-1}\)).

**Statistical analysis.** All data in the experiment are expressed as means ± SE. Differences between groups were determined by using Student’s t-test for independent means. A value of P ≤ 0.05 was considered statistically significant.

**RESULTS**

**Effects of chronic RUPP on systemic hemodynamics.** Figure 1A shows that chronic RUPP in pregnant rats resulted in a 33 mmHg rise in AP compared with the NP rats (132 ± 4 vs. 99 ± 3 mmHg, P < 0.001). The CO values presently observed in the NP dams are similar to those reported previously by our laboratory (19) and others (16, 29). CO was considerably decreased in the RUPP compared with the NP dams (104 ± 7 vs. 65 ± 5 ml/min; P < 0.05). Similarly, RUPP rats had 29% lower CI (246 ± 20 vs. 348 ± 19 ml·min\(^{-1}\)·kg\(^{-1}\), P < 0.002; Fig. 1B) than NP rats. However, Fig. 1C shows that TPR in RUPP rats was 119% higher (2.15 ± 0.03 vs. 0.98 ± 0.08 mmHg·ml\(^{-1}\)·min\(^{-1}\); P < 0.001) compared with NP rats.

**Changes in regional blood flow in response to chronic RUPP.** Placental blood flow was 60% lower in the RUPP rats (0.27 ± 0.08 vs. 0.7 ± 0.14 ml·min\(^{-1}\)·g\(^{-1}\) tissue; P = 0.05; Fig. 2A) compared with NP rats. Furthermore, total placental weight was decreased in the RUPP compared with the NP control rats (2.2 ± 0.2 vs. 4.8 ± 0.2 g; P < 0.001). This observation reflects the ~50% reduction in fetal number that accompanies the RUPP maneuver. In addition, blood flow to the uterus at day 18 of gestation was 54% lower in RUPP rats (0.16 ± 0.03 vs. 0.38 ± 0.09 ml·min\(^{-1}\)·g\(^{-1}\) tissue; P < 0.01; Fig. 2B) compared with NP rats.

The regional blood flows in different vascular beds in RUPP and NP rats at day 18 gestation are listed in Table 1. The RUPP maneuver resulted in significant reductions in blood flow to the heart (P < 0.04), stomach (P < 0.01), intestine (P < 0.04), and skeletal muscle (P < 0.05) when compared with the NP rats.
No differences were observed in blood flow to the brain, liver, kidney, or spleen.

**DISCUSSION**

The present study reveals several important findings regarding the systemic hemodynamics of the RUPP model of PIH. Foremost is that, much like what is observed in women with preeclampsia, the RUPP rat has decreased CO along with increased TPR and frank hypertension. Second, we report that both uterine and placental blood flow is decreased 60% and 54%, respectively. Last, we show that several vascular beds, both central and peripheral, receive reduced blood flow as a result of chronic RUPP.

Although the mechanisms underlying the pathophysiology of PIH remain controversial, placental ischemia is regarded to be a key factor (7, 26). Despite the consensus on this matter, to the best of our knowledge no previous studies have established the effects of impaired uterine perfusion on systemic hemodynamics during pregnancy. We have previously reported that the RUPP maneuver does not elicit increased MAP in the nonpregnant female rat (3). Thus the hypertension observed in the RUPP model is dependent on the presence of the placenta and possibly the hormonal milieu of pregnancy.

Clinical studies have resulted in the generation of a hyperdynamic model for cardiovascular function during preeclampsia that is characterized by a crossover from a high CO-low resistance state during the preclinical phase of the disease to a...
low CO-high resistance state during the clinical expression of the disease (8). Although it should be pointed out that these clinical findings may reflect increased body mass index in the preeclamptic group rather than simply existence of preeclampsia. Further studies are needed to clarify this matter. Our findings show that chronic RUPP result in a low CO-high resistance state that closely resembles the hemodynamic profile demonstrated by women with overt preeclampsia. Moreover, the present findings are in agreement with previous studies in preeclamptic women that indicates decreased CO and TPR is increased (8). Viewed in concert with our previous studies, these data clearly show widespread endothelial dysfunction is present in the RUPP hypertensive pregnant rat (3, 4, 12). Furthermore, our these findings are consistent with the notion of a clinically damaging, vasoconstricted state present in overt preeclampsia (8).

These data regarding CO and TPR are also in agreement with previous work employing Nω-nitro-L-arginine methyl ester (L-NAME) administration in the pregnant rat (19). Kassab et al. (19) showed that nitric oxide synthase inhibition (via L-NAME) in the pregnant rat also increased TPR while decreasing CO in late gestation. Viewed together with the present study, it appears that one or more factors emanating from the placenta as a result of RUPP may inhibit normal nitric oxide production during late gestation and alter normal systemic hemodynamics. Tumor necrosis factor-α is elevated in both preeclamptic women and the RUPP model in rats and is known to decrease neuronal nitric oxide synthase expression in the RUPP pregnant rat (2, 20). Similarly, endothelin and sFlt-1 are reported to be increased in both preeclamptic women and in the RUPP model of hypertension (4, 10, 14, 23). Furthermore, elevated plasma soluble fms-like tyrosine kinase-1 concentration has been associated with abnormal uterine artery pulsatility index, an often-used clinical proxy for uterine blood flow (13). Although it is evident that the ischemic placenta secretes numerous vasoactive factors, it remains unclear which of these factors or which combination of factors is responsible for the presently observed alterations in systemic hemodynamics.

As expected, in the present study, we found that uterine and placental blood flows were decreased ~60% in the RUPP compared with the NP dams. It is important to note that the presently observed reduced placental flow is per gram of placental weight. Thus the observed decrease in flow is not a function of the increased fetal and placental demise that is present in the RUPP model. Alternatively, it is likely that the increased number of resorptions and decreased conceptus mass observed in this model are effects of the decreased uteroplacental blood flow that we report in this study. Furthermore, we also report that chronic RUPP during pregnancy elicited reductions in blood flow to the heart, skeletal muscle, stomach, and intestine. The present findings share several similarities with previous work by Kassab and colleagues (19), who also reported decreased blood flow to cardiac and skeletal muscle while inhibiting nitric oxide synthase activity with L-NAME. In contrast to the present work, Kassab et al. (19) did not report reduced blood flow in the uteroplacental unit nor in the stomach or intestine. Furthermore, we did not observe significant reductions in renal, hepatic, and pulmonary blood flow previously reported in the L-NAME model of PIH (19). Taken together, the findings of the present study and that of Kassab et al. suggest that there are specific differences in the manner that vascular resistance in increased between these two models of PIH and that these differences may have important implications with respect to the utility of these models for elucidating the mechanisms of endothelial dysfunction in PIH.

In summary, the present study clearly shows that chronic RUPP in the pregnant rat generates cardiovascular adaptations that share robust similarities with the preeclamptic state observed in women. We report increased TPR and AP along with decreased CO and decreased uterine and placental blood flow, all of which closely mimic the clinical manifestations of preeclampsia. Our findings also demonstrate that there are differences in the vascular beds affected between the RUPP and L-NAME models of PIH. Last, these data further demonstrate the utility of the RUPP model for detailed study of the mechanisms underlying the pathology of preeclampsia.

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GRANTS

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REFERENCES
