Long-term effects of repeated pregnancies (multiparity) on blood pressure regulation

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Abstract

Objective: Pregnancy is associated with profound alterations in the cardiovascular system, the long-term effects of which are unknown. Human epidemiological studies suggest that multiparity (multiple pregnancies) increases the risk of cardiovascular disease. The mechanisms underlying these findings remain to be elucidated. The objective of this study was to determine the long-term effects of parity on cardiovascular regulation.

Methods: Pressor responses to phenylephrine (PE) and acute stress were compared in conscious age-matched repeatedly breed (RB) and virgin rats. Vascular compliance and reactivity of isolated resistance-sized mesenteric arteries were studied using pressure and wire myograph.

Results: We found that both exogenous PE and acute stress elicited greater pressor responses in RB than in aged-matched virgins. Pressure and wire myography also revealed that small mesenteric arteries from RB rats were less compliant than those from virgins (RB: 0.24±0.04 μm mm Hg⁻¹, n=6 vs. virgins: 0.63±0.06 μm mm Hg⁻¹, n=6; p<0.05) and were more sensitive to PE (EC₅₀ RB: 1.58±0.08×10⁻⁶ M, n=10 vs. virgins: 2.05±0.09×10⁻⁶ M, n=14; p<0.05). Removal of the endothelium abolished the difference in sensitivity. More specifically, the augmented vascular response of RB was both nitric oxide (NO) and cyclooxygenase dependent. By contrast, there was no difference in methacholine-induced vasodilation of phenylephrine-preconstricted vessels.

Conclusion: Our results suggest that repeated pregnancies induce long-term alterations in cardiovascular regulation due to changes in vascular compliance and endothelium-dependent vasoconstriction. We propose that such changes might influence the risk for cardiovascular disease in multiparous women.

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Keywords: Parity; Stress; Cardiovascular diseases; Vascular reactivity

1. Introduction

Cardiovascular disease is the leading cause of death and disability in women [1]. Epidemiological evidence suggests that multiparity (multiple pregnancies) may be a risk factor for cardiovascular disease [6,14,17,22,23]. There is also evidence from animal studies that repeated pregnancies can adversely affect the cardiovascular system. In rats, it has been reported that repeated pregnancies are associated with degradation of vascular elastic tissue [35] and an increase in the incidence of spontaneous arteriosclerosis of the aorta, and of the mesenteric and renal vascular beds [36,38]. It has also been reported that repeated pregnancies attenuate the production of nitric oxide (NO) in the kidney, which results in increased vasoconstriction in renal blood vessels [26].

Based on the above findings, the current study was conducted to explore the effects of repeated pregnancies on cardiovascular regulation. We hypothesized that multiparity would increase the pressor response to exogenous...
administration of the sympathomimetic phenylephrine (PE) and to endogenous sympathetic activation (stress). Furthermore, we proposed that such an augmented response would be mediated through increased constriction of the arterial resistance vasculature in the parous animals. The data support our hypotheses and point to there being long-term changes in both compliance and endothelial function of the mesenteric vascular bed of repeatedly bred animals.

2. Methods

The experimental procedures were approved by the local Animal Welfare Committee in accordance with the guidelines issued by the Canada Council on Animal Care, which conforms to NIH guidelines.

2.1. Animals and housing

Seven to 8-month-old female Long Evans (specific pathogen-free) retired breeder (RB) rats were obtained from Charles River, St Foy, Quebec, Canada. These animals had undergone five pregnancies, their age at first pregnancy being 56 days. They had been mated after weaning at 3 weeks postpartum. The control animals were aged-matched virgin rats, also from Charles River, which had been raised in the same living conditions as the retired breeders. After receiving the rats from Charles River, they were held in the University of Alberta animal facility on a 12-h–12-h light dark cycle, in a humidity and temperature-controlled environment, and allowed access to water and a 0.3% sodium diet ad libitum. A period of at least 1 month was allowed to elapse before they were used in the study.

2.2. Surgery

Rats were anesthetized with pentobarbital sodium (62 mg · kg⁻¹ body wt. i.p.), followed by atropine (0.1 ml, 0.4 mg ml⁻¹ s.c.). Buprenorphine (0.01 mg kg⁻¹ s.c.) was given after the completion of surgery. A nonocclusive cannula (Silastic, ID: 0.51-mm, OD: 0.94-mm) was implanted into the inferior vena cava for drug infusion [15]. A pressure transmitter (PA-C40, Data Sciences International) was implanted in the abdominal aorta [2]. Animals were allowed to recover for one week from surgery and to regain their preoperative body weight.

2.3. Pressor response to PE

After 1-day acclimatization to the telemetry cages, mean arterial pressure (MAP), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were continuously monitored in a stress-free environment in conscious, unrestrained rats using the PhysioTel Telemetry System (Data Sciences International) and later analyzed offline (Windaq, DATAQ Instruments). On the day of the experiment, baseline blood pressure was recorded for an hour. This was followed by short-term infusion (over 30 s) of 1, 3, 10 and 30 µg kg⁻¹ of PE; this protocol avoided acute baroreflex resetting [16]. A 10-min interval was allowed between each PE dose, which allowed the blood pressure and heart rate (HR) to return to baseline values. Baroreflex sensitivity, which is defined as ΔHR/ΔBP, was estimated at a dose of 10 µg kg⁻¹ PE. We found that HR was most stable after BP had fallen to 80% of its peak value. The relationship between HR and BP was thus measured at this point.

Fig. 1. Effect of parity on changes in (A) MAP, (B) SBP, (C) DBP in response to intravenous PE administration in conscious rats. Repeatedly breed rats: closed circles (n=6); virgins: open circles (n=7). Vertical lines delineate standard error of mean. *P<0.05, significant difference between repeatedly breed and virgin rats.
2.4. Blood pressure response to acute stress

Baseline blood pressure was recorded in conscious rats as described above. They were then exposed to acute stress by directing a jet of pressurized air towards them for 10 s. Changes in BP and HR were continuously recorded and changes in the MAP, SBP and DBP were compared.

2.5. Preparation of isolated vessels

The rats were decapitated and a segment of the small intestine and attached mesentery was isolated (~10 cm from the ileal-cecal junction). The vessels, arteries <250 μm in diameter and ~2 mm in length, were dissected in cold (0–4 °C) HEPES for wire myography (concentration in mmol l⁻¹: 142 NaCl, 4.7 KCl, 1.17 MgSO₄, 1.56 CaCl₂, 1.18 K₂PO₄, 10 HEPES and 5.5 glucose, at pH 7.4) or Dulbecco’s medium for pressure myography (concentration in mmol l⁻¹: 15 HEPES, 15 glucose, 1 sodium pyruvate, 25 sodium bicarbonate and 1 μM EGTA with 1 g l⁻¹ albumin (IgG and endotoxin free) at pH 7.4).

2.6. Vascular reactivity

Isolated mesenteric arteries were mounted on an isometric wire myograph system (Kent Scientific, Litchfield, CA, USA) and the constrictive responses to PE were measured in vessels as previously described [3]. To study the role of endothelium in the altered vascular reactivity, mesenteric vessels were divided into three groups: control vessels (not incubated with any drug), L-NAME-treated (incubated with L-NAME; nitric oxide synthase inhibitor, 100 μmol l⁻¹) and meclofenamate-treated (incubated with meclofenamate; cyclooxygenase blocker, 10 μmol l⁻¹). Vessels were incubated with L-NAME or meclofenamate for 20 min, after which (without washout) the constrictive response to PE was measured.

To assess endothelium-dependent vasorelaxation, isolated mesenteric vessels were first exposed to a cumulative concentration–response regime of PE to determine the concentration of PE required to produce 80% constriction (EC₈₀). The vessels were then pre-constricted with phenylephrine (EC₈₀) and methacholine concentration–response curves were completed.

2.7. Vascular compliance

The diameter-pressure relation of small mesenteric vessels was examined, by pressure myography (Living Systems Instrumentation, USA), using Ca²⁺-free Dulbecco’s medium bubbled with 95% air–5% CO₂ (Praxair) [8]. It has previously been reported that, in the pressure myograph system, this medium helps to maintain the physiological properties of the blood vessels [19]. Vessel diameter and wall/lumen ratio were displayed and measured using a CCD camera (Hitachi, Canada) and a video dimension analyzer (Living Systems Instrumentation).

![Fig. 2. Effect of parity on changes in (A) MAP, (B) SBP, (C) DBP and (D) HR in responses to stress (10 s air jet) in conscious rats. Repeatedly breed rats: closed circles (n=6); virgins: open circles (n=5). The horizontal bar shows the period during which stress was administered (10 s). Vertical lines delineate standard error of mean. *P<0.05, significant difference between repeatedly breed and virgin rats.](https://academic.oup.com/cardiovascres/article-abstract/64/1/179/297282)
2.8. Serum estradiol

Serum estradiol was measured using an Ultra-Sensitive Estradiol RIA kit with detection limit of 2.2 pg ml\(^{-1}\) (Diagnostics Systems laboratories). All samples were run in duplicate and the standard protocol supplied with the RIA kit was followed.

2.9. Pathology of the vascular tissue

Segments of mesenteric and renal arteries and aorta were dissected out from RB (\(n=8\)) and virgin (\(n=5\)) rats, and fixed with 10% formalin. The tissues were then processed for histological evaluation using hematoxylin and eosin staining (Health Sciences Laboratory Animal Services, University of Alberta).

2.10. Statistical analysis

Between-groups variation (retired breeders vs. virgins) was assessed using repeated measures two-way ANOVA, followed by post hoc analysis with the Student–Newman–Keuls test. In the stress response study (Fig. 2), each data point represents the mean changes in the blood pressure over the preceding period of 5 s. For simple comparisons between two sets of data such as EC\(_{50}\), we used Student’s \(t\)-test for unpaired data. All data are presented as mean±S.E. of mean. All results were considered statistically significant at \(p<0.05\).

3. Results

3.1. In vivo study

There was no significant difference between the resting MAP of the two groups, although it tended to be lower in the parous rats (RB: 98.7±2.4 mm Hg, \(n=12\) vs. virgin: 104.3±2.1 mm Hg, \(n=12\); \(p=0.09\)). Nor were there any significant differences between the groups with regard to systolic (SBP; RB: 117.0±2.9 mm Hg, \(n=12\); \(p>0.05\)) or diastolic (DBP; RB: 86.8±2.3 mm Hg, \(n=12\) vs. virgin: 91.7±2.0 mm Hg, \(n=12\)).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>RB (n=10)</th>
<th>ED (n=5)</th>
<th>Virgin (n=14)</th>
<th>ED (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC(_{50}) (10(^{-6}) M)</td>
<td>1.58±0.08*</td>
<td>1.05±0.23</td>
<td>2.05±0.09</td>
<td>1.08±0.14</td>
</tr>
<tr>
<td>Max tension (mN mm(^{-1}))</td>
<td>0.38±0.01</td>
<td>0.31±0.03</td>
<td>0.38±0.01</td>
<td>0.32±0.03</td>
</tr>
</tbody>
</table>

Date presented as mean±standard error of mean.

* \(P<0.05\), significant difference between RB and virgin rats.

### Table 2

<table>
<thead>
<tr>
<th>Tension (mN mm(^{-1}))</th>
<th>RB (n=6)</th>
<th>Virgin (n=7)</th>
<th>RB (n=5)</th>
<th>Virgin (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>0.075±0.013</td>
<td>0.083±0.008</td>
<td>0.084±0.013</td>
<td>0.062±0.065</td>
</tr>
<tr>
<td>After</td>
<td>0.073±0.012</td>
<td>0.081±0.009</td>
<td>0.083±0.013</td>
<td>0.060±0.063</td>
</tr>
</tbody>
</table>

Date presented as mean±standard error of mean.
n=12; p≥0.05) pressures. However, the pressor response to PE (MAP, SBP and DBP) was significantly greater in RB animals than in the age-matched virgin control rats (Fig. 1). There was no significant difference in baroreflex sensitivity between the two groups (AHR/AHR; RB: 2.76±0.86 bpm mm Hg, n=6; virgin: 2.80±0.48 bpm mm Hg, n=7; p≥0.05). Stress elicited an immediate increase in MAP, which was significantly greater in the RB group (Fig. 2A). There was also a significantly greater increase in both SBP and DBP in RBs compared to virgin rats (Fig. 2B and C). There was no significant difference in the stress-induced change in HR of the RB and virgin rats (Fig. 2D).

3.2. Vascular reactivity

3.2.1. Effects of parity on mesenteric artery contractile responses

Mesenteric arteries from the RB were more sensitive to PE than those from the virgin animals, although the maximum responses did not differ (Fig. 3A, Table 1). Removal of the endothelium shifted the concentration–response curves of both RB and virgin rats leftwards, such that the difference between the two groups was abolished (Fig. 3A, Table 1).

3.2.2. Nitric oxide and cyclooxygenase dependent modulation of contractile responses

Incubation of mesenteric arteries with l-NAME or meclofenamate had no affect on the baseline tensions of either RB or virgin rats (Table 2). Similarly, pretreatment with l-NAME did not alter the maximum PE-induced vasoconstriction of mesenteric arteries from either RB or virgin rats. However, the concentration–response curves from both groups were shifted leftward, such that the difference in PE sensitivity between them was abolished (Fig. 3B, Table 3). Meclofenamate also shifted the concentration–response curves, this time to the right. Again, the difference in PE sensitivity between the parous (RB) and virgin groups was abolished (Fig. 3C, Table 3). Like l-NAME, meclofenamate did not alter the maximal responses to PE (Fig. 4, Table 3).

3.2.3. Effects of parity on endothelium dependent vasorelaxation

Although endothelium dependent vasorelaxation to methacholine tended to be attenuated in RB (EC50 RB: 5.37±1.29×10⁻⁶ M, n=8) compared to virgins (EC50 virgin: 3.64±1.29×10⁻⁶ M, n=9), it failed to reach significance (p=0.360).

3.3. Vascular compliance

In both RB and virgin rats, the diameter of small mesenteric arteries increased with increasing intraluminal pressure. However, at physiological pressures (80–120 mm Hg), the ratio of change in diameter: change in pressure was significantly lower in arteries form RB than from age-matched virgins rats (RB: 0.24±0.04 mm mm Hg⁻¹, n=6 vs. virgin: 0.63±0.06 mm mm Hg⁻¹, n=6; p=0.05) (Fig. 5).
Rennke [5] found resting MAP to be higher in repeatedly breed (RB) rats compared to virgins [26]. By contrast, Baylis and Rennke [5] found no evidence for atherosclerotic lesions identified in any of the vessels from either the RB or virgin rats.

### 3.4. Serum estradiol levels

There was no significant difference in serum estradiol levels between the RB (22.06 ± 3.61 pg ml⁻¹, n = 16) and virgin (23.58 ± 4.88 pg ml⁻¹, n = 15) rats (Fig. 6).

### 3.5. Vascular morphology

There was no difference in the wall/lumen ratios of the two groups (RB: 0.27 ± 0.01, virgins: 0.28 ± 0.08). Nor were atherosclerotic lesions identified in any of the vessels from either the RB or virgin rats.

### 4. Discussion

Multiparous (RB) rats responded to acute stress and to exogenous PE with a greater increase in blood pressure than did the virgin animals. Despite the enhanced pressor responses to acute stimuli, resting MAP tended to be lower in the RB than in virgin rats, although this did not reach significance. A lower resting blood pressure in RB was also reported by Reckelhoff [26]. By contrast, Baylis and Rennke [5] found resting MAP to be higher in repeatedly bred rats. Given our finding that the pressor response to stress is greater in parous rats, this discrepancy may be attributed to the status of the animal during the experiment, e.g., the depth of anesthesia, the anesthetic agent and the fluid/electrolyte balance. It was for this reason that we chose to measure blood pressure in fully recovered, unrestrained, conscious rats.

The pressor response to exogenous PE was greater in the RB rats than in the virgins. Since this difference was apparent in both the SBP and DBP pressure traces, we suggest that the augmented pressor response to exogenous PE can be attributed primarily to increased total peripheral resistance rather than to increased cardiac output [18,28]. The magnitude of the pressor response to exogenous PE depends, not only on the sensitivity to vasoconstriction of the arterial resistance vessels (increase in TPR), but also on the degree of buffering by the baroreflex. We found no difference in baroreflex gain between the RBs and the virgins (ΔHR/ΔBP). This further supports our contention that the greater pressor response of the parous animals was caused by a greater increase in TRP in RB due to an enhanced sensitivity of their vasculature to PE. Again, our in vitro studies confirmed this. Resistance-sized mesenteric arteries from parous rats had a greater endothelium-dependent constriction to PE than those from virgin animals.

Stress induces a sympathetically mediated increase in cardiac output, as well as vasoconstriction (particularly in the mesenteric vascular bed) [42]; this results in an increase in MAP. Parous rats had a potentiated pressor response (MAP) when exposed to acute stress initiated by a jet of pressurized air. This was associated with parallel responses in both SBP and DBP, again suggesting a greater increase in total peripheral resistance in RB rats. Furthermore, given that there was no difference between the RB and virgin rats with respect to change in heart rate, we can probably assume that the magnitude of the stress-induced increase in autonomic outflow was similar in the two groups. Thus, the enhanced response of the RB rats may be ascribed primarily to a greater increase in total peripheral resistance. This would again be consistent with our finding that isolated resistance-sized vessels from parous rats were more responsive to PE than were those from virgin rats.

We studied mesenteric vessels because the splanchnic vascular bed plays a major role in determining the total vascular resistance as well as blood distribution within the intravascular space [25]. Thus, any changes in the active (vascular reactivity) and/or passive (vascular compliance) properties of the splanchnic blood vessels may significantly affect overall cardiovascular homeostasis.

Isolated mesenteric arteries from RB rats were more sensitive to PE than were those from virgin animals. This difference in PE sensitivity was endothelium-dependent, since removal of endothelium abolished the difference. Endothelium-dependent a-adrenergic constriction in arteries can be modulated by the NO and cyclooxygenase pathways [39]. To investigate the mechanisms by which PE sensitivity was increased in parous rats, we blocked these pathways with L-NAME and meclofenamate respectively. L-NAME caused a significant increase in PE sensitivity in virgin rats, but not in parous rats, so that there was no longer any difference between the two groups. Meclofenamate also abolished the difference between the two groups, this time by attenuating the sensitivity of the vessels to PE. These data suggest that repeated pregnancy blunts the activity of the vasodilatory NO system and enhances the production of vasoconstrictive prostaglandins. Increased vasoconstriction and attenuation of NO production have also been reported in the renal vasculature of repeatedly breed (RB) rats [26]. Indeed, Reckelhoff [26] has suggested that pregnancy might leave the vessels with some degree of endothelial damage, which could ultimately cause endothelial dysfunction. By contrast, Baylis and Rennke [5] found no evidence for
structural or functional abnormalities in the renal vasculature. Reckelhoff attributed this to a species-dependent difference in the effect of aging on the renal vasculature. Interestingly, we did not find any significant difference in endothelium-dependent vasorelaxation of the RB and virgin rats. This suggests that parity primarily alters vasoconstriction through modulation of adrenergic-dependent NO and cyclooxygenase pathways, rather than by altering endothelium-dependent vasodilation per se.

We found that a rise in intraluminal pressure caused a smaller increase in the diameter of vessels from parous rats compared with those from virgins, i.e., they were less compliant. This is consistent with previous reports of degradation of vascular elastic tissue in repeatedly bred female rats [35,36,38]. The decrease in vascular compliance would make the vessels stiffer and the cardiovascular system less able to compensate for any fluctuations in blood pressure. Furthermore, augmented reactivity to α-adrenomimetics would enhance the response to an increase in sympathetic outflow. This is exactly what we found in our in vivo studies.

The mechanisms underlying the effect of repeated pregnancies on vascular compliance and endothelium function remain uncertain. This could be due to resetting of the hormonal system of the animal after pregnancy. It has been reported that pregnancy permanently reduces plasma levels of prolactin [33,41] and estrogen [7]. Both of these hormones have well-established effects on the cardiovascular system. Estrogen has been shown to increase NO biosynthesis, to attenuate arterial vasoconstriction to PE, angiotensin and vasopressin, and to increase vascular compliance [4,11,32]. Furthermore, prolactin mRNA and receptors have recently been identified in endothelial and smooth muscle cells [9], suggesting that it too can act in an autocrine/paracrine manner within blood vessels. Although we did not find any difference in the serum estradiol levels between RB and virgin rats, we cannot discount the role of this hormone entirely, since repeated pregnancy has also been reported to significantly reduce the population of estrogen receptor (ER) positive cells, at least in the mammary glands [29,40].

Parity-induced alterations in the vascular system may also be attributed to enhanced oxidative stress. Pregnancy is associated with an increase in low-density lipoprotein (LDL), total cholesterol and triglycerides levels [13,14]. Some of these changes in the lipid profile persist postpartum, long after reproductive activity has ceased [14]. Hyperlipidemia, especially high LDL, is responsible for the formation of oxidizable particles, increased oxidative stress and atherosclerosis leading to endothelium dysfunction and decreased vascular distensibility [12,20,25,30,31]. This is what we found in our study where vessels for parous rats were less compliant and more sensitive to phenylephrine. Interestingly, parity has been reported to be associated with increased incidence (number and severity) of atherosclerotic plaques in both rats and humans [14,36], and with degenerative changes in vascular elastic tissues and decreased collagen content [34,36–38]. Although our histological studies failed to detect any gross atherosclerotic changes in either RB or virgin rats, we stained only with hematoxylin and eosin. Specialized stains such as those used by Wexler would have been required in order to reveal alterations in elastic tissue and ground substance [36,37].

The high incidence of atherosclerosis in the rats used in previous studies may be linked to the pathogen status of those rats. There is increasing evidence that infectious agents can play an important role in development of atherosclerosis or may intensify the effects of other risk factors [10,21]. Most of the previous studies examining the relationship between parity and atherosclerosis were carried out 40–50 years ago, when laboratory rats were not pathogen free. By contrast, the rats used for these current experiments were specific pathogens free (SPF rats from Charles River, St Foy, Quebec, Canada). This might explain the reason why none of our rats had any frank signs of atherosclerotic lesions. Furthermore this does also raise the question as to whether, if parous animals are exposed to risk factors (such as infectious agents), they might have a higher probability of developing cardiovascular disease than non-parous animals.

It is difficult in epidemiological studies to control for potentially confounding factors such as socioeconomic status and the psychological stresses of child rearing [23]. Thus, well-controlled animal studies are critically important. Not only are the pregnancy-associated changes observed in rats are very similar to those found in humans [27], but we can induce repeated pregnancies within a short interval of time. Thus, in the rat, we have been able to show that repeated pregnancy augments the pressor response to intravenous infusion of PE and to acute stress due, at least in part, to changes in both the passive and active characteristics of the mesenteric blood vessels. This could lead to transient stress-induced increases in total peripheral resistance and cardiac afterload which, in time, could contribute to the increased risk for cardiovascular disease observed in multiparous individuals.

Acknowledgments

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References


