Adult Rabbit Offspring of Mothers With Secondary Hypertension Have Increased Blood Pressure

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Abstract—Preexisting chronic hypertension complicates up to 5% of pregnancies and is associated with an increased risk of low-birth-weight babies. Studies suggest that an adverse intrauterine environment leading to low birth weight is linked to an increased risk of cardiovascular disease, including hypertension, in the adult. In this study, the blood pressure of offspring from mothers with hypertension were followed up into adulthood. Two-kidney, 1-wrapped hypertension was induced in 7 female rabbits; 5 other rabbits underwent sham surgery. Four weeks later, rabbits were mated, at which time mean arterial pressure was 118±3 and 87±5 mm Hg in the hypertensive and sham groups, respectively (P<0.001). The blood pressure of 30-week-old females was 89±2 mm Hg in the offspring of hypertensive (n=14) and 79±1 mm Hg in the offspring of normotensive (n=13) mothers (P<0.005). Also, plasma renin activity was significantly lower in the female offspring of hypertensive mothers at 10 weeks of age (P<0.05), suggesting that development of the renin-angiotensin system was altered. In contrast, male offspring from hypertensive and normotensive mothers had similar mean arterial pressure and plasma renin activity. In conclusion, maternal secondary hypertension can “program” hypertension in female adult offspring. The results also suggest that there are gender-specific differences in sensitivity to altered in utero environmental influences. (Hypertension. 2003;41[part 2]: 000-000.)

Key Words: blood pressure ■ rabbits ■ hypertension, secondary ■ pregnancy ■ renin ■ gender

Hypertension is the most common complication associated with pregnancy.1 Preexisting chronic hypertension complicates up to 5% of pregnancies and is associated with an increased risk of preterm labor, uteroplacental insufficiency, placental detachment, and low birth weight.2,3 In the United States, the percentage of mothers older than 30 years increased from 16% in 1976 to 37% in 20014,5; it is also known that the rate of hypertension increases in women >30 years of age.6 It is therefore predicted that the incidence of chronic hypertension during pregnancy will increase in association with this trend toward childbearing at older ages. Epidemiological studies suggest that an adverse intrauterine environment leading to low birth weight is linked to an increased risk of cardiovascular disease, including hypertension, in the adult.7,8 Even mild chronic hypertension during pregnancy has been associated with low birth weight.2,3 We have therefore investigated the effect of chronic hypertension during pregnancy on the blood pressure of adult offspring. To this end, secondary hypertension was induced in female rabbits, these rabbits were mated, and then the blood pressure of their offspring was followed up into adulthood. The adult offspring were also subjected to the prohypertensive physiological challenges of an increased salt diet and weight gain.

Methods

Animals

English crossbred rabbits were used. Experiments were approved in advance by the Monash University Standing Committee on Ethics in Animal Experimentation.

Mothers

Female rabbits were fed ad libitum (pellets/lucerne/oat chaff in the ratio of 4:1:1 by weight). On day 0, mean arterial pressure (MAP) and body weight were measured in rabbits, and blood was collected (1.5 mL) to measure hematocrit and plasma renin activity (PRA).9 Surgery was then performed to induce 2-kidney, 1-wrapped (2K,1W) Page hypertension (n=7; mean±SEM age, 4.3±0.5 months; mean±SEM weight, 3.2±0.1 kg). The control group underwent a sham operation (n=5; mean±SEM age, 4.3±0.9 months; mean±SEM weight, 3.2±0.2 kg). In brief, anesthesia was induced with propofol (10 mg/kg IV; Diprivan, Zeneca) and maintained with isoflurane (Forthane, Abbot). The left kidney was exposed through a flank incision, gently cleared of fat, and wrapped in cellophane (15×12 cm, Clearpack); the right kidney remained untouched.10,11 Exactly the same procedure was followed for the sham operation: the flank incision was made, but the kidney was not wrapped in cellophane. MAP, body weight, hematocrit, and PRA were measured 2 and 4 weeks after surgery, and the rabbits were then mated. After weaning, the mothers were killed and the kidneys examined. Seven litters from hypertensive mothers (n=14 female offspring, n=14...
male offspring) and 5 litters from normotensive mothers (n=13 female offspring, n=9 male offspring) were included in the study.

**Fathers**

Four normotensive male rabbits (mean±SEM age, 13±3 months) unrelated to the mothers were randomly used to impregnate the females.

**Offspring**

Offspring were weaned at 5 weeks of age, and between 5 and 10 weeks, the rabbits were fed pellets (Barastoc rabbit and guinea pig pellets, Ridley Agriproducts, Australia) ad libitum; from 10 weeks of age, food was restricted to 100 g of the pellet/chaff mixture per day. The NaCl content of this mixture was ~0.85 g/100 g of diet. All rabbits ate the entire 100-g food allocation each day. Standard measurements were taken each time that the offspring were examined. These included MAP, body weight, hematocrit, and PRA. Measurements were taken at 10 (adolescent) and at 30 (adult), 34, 40, and 44 weeks of age. Rabbits reach sexual maturity at ~14 weeks of age.

**High-Salt Diet**

At 30 weeks of age, the salt diet of the offspring was increased from 0.85 g to 5 g per 100 g of food. As stated previously, each rabbit received 100 g of food per day, and all rabbits, including those feed the high-salt diet, ate the full allocation each day. After 4 weeks, the standard set of measurements was repeated.

**Increased Food Intake**

At 34 weeks of age, the offspring’s normal diet (0.85 g NaCl per 100 g of food per day) was reestablished for 6 weeks, and standard measurements were repeated at 40 weeks of age. Offspring were then given unrestricted access to the pellet/chaff mixture, and food intake was increased from 100 g per day to ~300 g per day. After 4 weeks, at 44 weeks of age, the standard set of measurements was repeated. Offspring were then humanely killed (1200 mg sodium pentobarbital, Lethabar, Virbac Pty Ltd), and the kidneys were weighed.

**Measurement of MAP**

MAP in conscious rabbits was measured with an ear artery catheter as previously described.21 MAP was continuously recorded for 30 minutes, with the mean of the final 15 minutes taken as the conscious resting pressure.

**Statistics**

Data are expressed as mean±SEM. Two-way repeated-measures ANOVA was used to test for statistical significance, the factors being time (Ptime) and group (Pgroup). The interaction term (Ptime*group) tested whether the response over time differed between the groups. Kidney weight data were analyzed by an unpaired t test. The software package SYSTAT was used (Systat Inc). P≤0.05 was considered statistically significant.

**Results**

**Mothers**

Blood pressures in the 2 groups of prospective mother rabbits were not different before the operation to induce hypertension (Figure 1). Blood pressure had increased in both groups at 4 weeks after surgery (Ptime<0.001), with MAP in the 2K,1W group increasing by 42±3 mm Hg compared with the sham-operated group, in which blood pressure increased 8±5 mm Hg (Ptime*group<0.001, Figure 1). Both groups had similar control levels of PRA; however, at 4 weeks after surgery, PRA was 14.3±2.8 and 1.6±0.5 ng angiotensin I (Ang I) · mL plasma⁻¹ · h⁻¹ in the 2K,1W and sham groups, respectively (Pgroup<0.01, Figure 1). Hematocrit did not differ between the groups. Both groups of rabbits gained weight with age (Ptime<0.001), but the normotensive rabbits (+350±90 g) had gained more than the hypertensive rabbits (+140±50 g) 4 weeks after surgery (Ptime*group<0.02, Figure 1). It was at this time that the female rabbits were mated.

All pregnancies went full term, 32±1 days for both hypertensive and normotensive mothers (Pgroup=0.7). There was no difference in litter size between hypertensive (6±1 kittens) and normotensive (4±2 kittens) mothers (P=0.2). The male-to-female birth ratio was 1:1 in the offspring of hypertensive (OHM) and normotensive (ONM) mothers (Pgroup=0.5). There was an average of 3±1 and 1±1 deaths per litter in the OHM and ONM groups, respectively (P=0.08). The majority of deaths occurred because the primigravidae mothers did not care for their kittens.

**Offspring**

Analysis of the data demonstrated a gender bias; therefore, the data are reported separately for female and male offspring.

**Females**

MAP in conscious rabbits increased from 76±2 to 89±2 mm Hg in the OHM (n=14) and from 76±2 to 79±1 mm Hg in the ONM (n=13) groups at 10 to 30 weeks of age, respectively. Blood pressure increased with time in both groups during the 20-week period but rose by a significantly greater extent in the OHM animals.
(13±2 mm Hg) compared with the ONM animals (3±2 mm Hg, \( P_{\text{time}^{*}\text{group}}=0.003 \), Figure 2). Heart rate decreased with age (\( P_{\text{time}}<0.001 \)), but the fall was similar in the 2 groups (\( P_{\text{time}^{*}\text{group}}=0.3 \)). There was no significant difference in hematocrit at 10 or 30 weeks of age in either group. PRA was 3.7±0.6 ng Ang I · mL plasma \(^{-1} \) · h \(^{-1} \) and 2.0±0.3 ng Ang I · mL plasma \(^{-1} \) · h \(^{-1} \) in the OHM group compared with 5.6±0.5 ng Ang I · mL plasma \(^{-1} \) · h \(^{-1} \) and 2.0±0.4 ng Ang I · mL plasma \(^{-1} \) · h \(^{-1} \) in the ONM group at 10 and 30 weeks, respectively (Figure 2). As expected, PRA fell as the rabbits aged in both groups (\( P_{\text{time}}=0.001 \)), but the rate of decrease in PRA was less in the OHM group (\( P_{\text{time}^{*}\text{group}}=0.05 \), Figure 2) because PRA was significantly lower at 10 weeks of age in the OHM group. Offspring body weight increased in both groups (\( P_{\text{time}}=0.001 \)) by similar amounts (\( P_{\text{time}^{*}\text{group}}=0.2 \), Figure 2).

**High-Salt Diet**

MAP was 92±2 and 90±2 mm Hg in the OHM group (\( n=9 \)) and 77±2 and 79±2 mm Hg in the ONM group (\( n=7 \)) before and after 4 weeks of the high-salt diet, respectively (\( P_{\text{time}^{*}\text{group}}=0.1 \), Figure 3). In the OHM group, PRA was 1.2±0.2 ng Ang I · mL plasma \(^{-1} \) · h \(^{-1} \) before and 1.0±0.3 ng Ang I · mL plasma \(^{-1} \) · h \(^{-1} \) after increased salt intake. In the ONM group, PRA was 1.4±0.4 ng Ang I · mL plasma \(^{-1} \) · h \(^{-1} \) before and 1.3±0.3 ng Ang I · mL plasma \(^{-1} \) · h \(^{-1} \) after increased salt intake (Figure 3). Body weight was not different between the groups (Figure 3).

**Increased Food Intake**

Body weight increased in both groups by 290±20 g in the OHM and by 210±40 g in the ONM (\( P_{\text{time}}<0.001 \)); the increase in body weight was not different between groups (\( P_{\text{time}^{*}\text{group}}=0.1 \), Figure 4). MAP was 90±2 and 96±2 mm Hg in the OHM (\( n=8 \)) and 80±2 and 78±2 mm Hg in the ONM at 40 weeks of age and at 44 weeks of age after 4 weeks of unrestricted access to food, respectively. MAP rose significantly in the OHM (7±3 mm Hg) compared with ONM (−2±1 mm Hg) animals during this period (\( P_{\text{time}^{*}\text{group}}=0.04 \), Figure 4). PRA increased during the 4-week period (\( P_{\text{time}}=0.02 \)), but the increase was similar in both groups (\( P_{\text{time}^{*}\text{group}}=0.9 \), Figure 4). At autopsy, total kidney wet weight was not significantly different, being 20.6±0.3 g in the OHM and 20.1±0.6 g in the ONM groups (\( P=0.8 \)).

**Males**

MAP in conscious rabbits increased from 78±1 to 83±2 mm Hg in the OHM (\( n=14 \)) and from 77±1 to 82±1 mm Hg in the ONM (\( n=9 \)) groups at 10 to 30 weeks of age, respectively. Blood pressure increased with time in both groups between 10 and 30 weeks of age, but the increase was not significantly different (Figure 2, \( P_{\text{time}^{*}\text{group}}=0.9 \)). Heart rate increased with age (\( P_{\text{time}}=0.001 \)), but the fall was similar in the 2 groups (\( P_{\text{time}^{*}\text{group}}=0.4 \)). There was no significant difference in hematocrit at 10 or 30 weeks of age in either group. In males, PRA was 4.4±1.2 ng Ang I · mL plasma \(^{-1} \) · h \(^{-1} \) and 2.6±0.4 ng Ang I · mL plasma \(^{-1} \) · h \(^{-1} \) in the OHM animals compared with 6.0±1.4 ng Ang I · mL plasma \(^{-1} \) · h \(^{-1} \) and 3.6±0.5 ng Ang I · mL plasma \(^{-1} \) · h \(^{-1} \) in the ONM animals at
MAP was 86 ± 3 mm Hg and 87 ± 2 mm Hg; n = 9) and ONM (85 ± 2 vs 90 ± 2 mm Hg; P_{time/group} = 0.6, Figure 4). PRA increased in the male rabbits with increased food intake (P_{time} = 0.05), but the increase was not different in the 2 groups (P_{time/group} = 0.2, Figure 4). At autopsy, total kidney wet weight was not significantly different, being 21.5 ± 1.2 g in the OHM and 21.9 ± 1.2 g in the ONM (P = 0.5).

**Discussion**

The female offspring of rabbit mothers with secondary renal hypertension had increased blood pressure as adults. In humans, chronic increases in blood pressure of similar magnitude (10 mm Hg) significantly increase the risk of cardiovascular disease.13 Our study therefore supports the hypothesis that an adverse intrauterine environment can alter fetal programming and lead to increased risk of hypertension.14,15

Interestingly, blood pressure in the male offspring of hypertensive mothers was unaffected. This suggests that males were "protected" or that the females were more susceptible to the adverse influence of chronic hypertension during pregnancy. It has been suggested that critical periods in development occur earlier in males, such that males grow earlier than females.16 A number of other studies have also clearly shown that there are gender-based differences in the response to adverse prenatal events in adults.17-21 One possibility is that these gender-specific responses are based on differences in prenatal programming of the hypothalamic-pituitary-adrenocortical axis. Additionally, we have previously shown that there are gender differences in the pressure-natriuresis relation in anesthetized male and female rabbits, the relationship being shifted to the right in females. There was, however, no effect on long-term blood pressure.12 Furthermore, gender-specific differences in renal responses to Ang II have been demonstrated in humans.22 These results suggest that there are differences in normal male and female renal function, and therefore, an adverse intrauterine environment may have different effects in males and females such that long-term blood pressure regulation is altered.

There are several maternal factors that may have contributed to female offspring’s having an increased arterial pressure in adulthood. The mothers, as well as being hypertensive (~30 mm Hg), also had reduced weight gain, elevated PRA (≈800%), and a degree of renal impairment.10,23 A study in which maternal hypertension was induced by infusion of aldosterone, 11α-hydroxyprogesterone, or carbenoxolone and showed no subsequent alteration in fetal development or pup blood pressure24 suggests that it was not the elevation in maternal blood pressure per se that was responsible for the increased blood pressure in the female offspring.

Uterine blood flow is known to be significantly reduced in mothers with existing chronic hypertension.25,26 and this can result in decreased delivery of nutrients, increased risk of hypoxemia, and other adverse effects in the fetus.3 For example, maternal corticotropin-releasing hormone levels have been shown to be elevated in chronic hypertension early in pregnancy.27 In sheep, early exposure to excess glucocorticoids during gestation leads to fetal growth retardation and hypertension in later life.28

It is a strong possibility that the fetal renin-angiotensin system (RAS) was perturbed. Certainly, the RAS was acti-
vated in the model used to induce hypertension in the mothers, and PRA was suppressed in the adolescent female OHM rabbits at 10 weeks of age. The importance of these findings remains to be investigated. It is known that the RAS plays a major role in development of the fetal cardiovascular system, particularly of the kidney.29,30 and has been implicated in undernutrition models of perinatal programming.21 Recently, a maternal low-sodium diet associated with increased maternal PRA has been shown in rats to result in intrauterine growth restriction, increased blood pressure, and reduced creatinine clearance in female offspring.19

The maternal influence on offspring blood pressure may not necessarily occur in utero. For example, offspring may have been affected by the quality or quantity of milk produced by the hypertensive mothers, but this is not supported in this study, because there were identical growth rates in the 2 groups. It is also possible the hypertensive mothers were secreting a prohypertensive substance in their milk or influencing their offspring’s blood pressure development by their feeding methods, as previously suggested.31,32 Several maternal factors, however, can be excluded as contributors to the increase in blood pressure, because all mothers were primigravidae and delivered at term, with litter sizes and gender ratios of offspring being similar, and the same normotensive males were the fathers in both groups.

We posed 2 physiological challenges to determine whether the increase in arterial pressure in the offspring of hypertensive mothers could be exacerbated by environmental factors. The first challenge was a high-salt diet; the second, increased food intake. We found that a high-salt diet did not affect blood pressure in the hypertensive or normotensive offspring in the experimental setting of the present study, although the elevation in blood pressure in the female OHM persisted throughout treatment. A previous study has shown that high salt intake does not affect blood pressure in rabbits.14 Therefore, either the OHM, or normotensive female offspring, were insensitive to high salt intake or the dietary change in salt content was not sufficiently severe. However, during the 4-week period when food intake was increased ≈3-fold and all of the rabbits gained weight, blood pressure was seen to increase in the OHM but not the ONM females, with the difference in MAP at 44 weeks of age between the OHM and ONM being ≈18 mm Hg. Blood pressure in the male offspring increased in response to increased food intake but was not significantly different between the normotensive and hypertensive groups. Interpretation of these results should be viewed with caution, because no control groups were included in the experimental design. Therefore, the greater rise in blood pressure in response to a 4-week period of increased food intake could be attributable to the weight gain or age-related changes. This finding warrants further investigation into glucose handling and the lipid profile in these rabbits.

Perspectives

Maternal secondary hypertension can “program” hypertension in adult offspring, increasing the potential risk of cardiovascular disease. The causes are not known, but the suppression of PRA in the adolescent females of hypertensive mothers suggests that further investigation of a possible role for altered fetal programming of the RAS in the elevation of adult blood pressure is warranted. The fact that blood pressure increased in female offspring only suggests that there are gender-specific differences in sensitivity to altered in utero environmental influences. If these results are relevant to humans, women with secondary hypertension (eg, from renal disease) may be at risk of giving birth to babies that may be at increased risk of developing hypertension as adults.

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References


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