Modulation of Blood Pressure in the Dahl SS/jr Rat by Embryo Transfer

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Abstract—Gestational hypertension and malnutrition are associated with hypertension and ischemic heart disease in the adult human. The impact of the gestational environment on the adult blood pressure in two well-characterized genetically homogeneous rat strains, the hypertensive SS/jr and normotensive SR/jr, was studied by cross-fostering within 6 hours of birth and by embryo transplantation with the recipient dam nursing the transplanted pups. Systolic blood pressure (BP) was measured by tail-cuff plethysmography twice a week after the age of 7 weeks. The lactational environment (cross-fostering) had no effect on blood pressure. Embryo transfer between like strains had no effect on the development of hypertension, nor did the BP of R transferred to S (RetS) differ from that of normal R or RetR. At 7 weeks of age, the BP of SetR was significantly lower than that of S or SetS (P < 0.01) and was similar to that of RetR and R. With age, the blood pressures of the S, SetS and SetR increased at approximately the same rate but from a significantly different baseline. Salt-sensitivity in the S and resistance in the R were not altered. The protective effect of the R gestational environment on SetR female BP was abrogated during whelping and lactation. Embryo transfer and cross-fostering did not alter the weight of rats older than 7 weeks. Because the BP of the R dams were significantly lower than that of the S dams, these studies do not distinguish between the effects of the R dams' lower blood pressure per se and hormonal influences of the R uterus on the S blood pressure phenotype. (Hypertension. 1998;31[part 2]:540-545.)

Key Words: hypertension ■ gestation ■ fetal environment ■ Dahl salt-sensitive rat

The causes of essential hypertension are complex, multifactorial, and interactive and can be broadly classified as genetic and environmental. There is epidemiological evidence in humans that gestational effects are important in blood pressure “imprinting,” that is to say, the level of blood pressure that will be “normal” for the individual as an adult. Low birth weight with a larger than normal placenta has been found to be a significant risk factor for cardiovascular disease, including hypertension, later in life. In another study that followed individuals encompassing ages 5 to 37 years for an average of 14 years, birth weight was inversely correlated with systolic blood pressure from childhood to young adulthood and with diastolic blood pressure in adults. Blood pressure was independent of gestational age. The combination of low birth weight and high current body mass index had the highest correlation with hypertension even in youngsters.

Berecek and collaborators have reported that the progeny of SHR, dams whose blood pressures were normalized by treatment with the converting enzyme inhibitor captopril, had significantly lower blood pressures than those of control SHR. Moreover, the effect persisted in the second generation, pups of the progeny whose dams had received the converting enzyme inhibitor also had lower blood pressures. The conclusion of these authors was that the gestational blood pressure, not ACE inhibition, was responsible for the long-term effects.

To test the hypothesis that the gestational milieu is an important environmental factor influencing the genetically determined blood pressure in the SS/jr rat, reciprocal embryo transfers between the hypertension-prone SS/jr and hypertension-resistant SR/jr were made, and the blood pressures of the progeny and their progeny from normal gestations were measured. Rats that had been reciprocally cross-fostered were studied to determine the effect of nurturing and lactational environment on blood pressure.

Methods

The Dahl SS/jr and Dahl SR/jr rats used in these experiments were generously provided by John Rapp of the Medical College of Ohio in 1984 and have been maintained as closed colonies under conventional husbandry conditions in AAALAC-accredited facilities. These experiments were done under approved protocols of both the University of Missouri–Columbia ACUC and the Harry S Truman Memorial VA Subcommittee on Animal Studies. Blood pressures have been measured in a large number of individuals throughout the years to ensure that there has been no genetic drift away from the hypertensive trait. A high salt intake greatly exacerbates the hypertension in SS/jr, however, given enough time, even on a normal-salt diet (0.28% sodium, Purina Formulab Chow), these rats eventually become extremely hypertensive and die of cerebrovascular, cardiac, or renal sequelae of hypertension. Since our SS/jr have a 100% incidence of cataracts that form during fetal life, the genotypes of the SS/jr and SR/jr are easily distinguished as soon as the eyes open.

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Neonates or embryos were transferred from SS/Jr to SR/Jr, from SS/Jr to SS/Jr, from SR/Jr to SS/Jr, and from SR/Jr to SR/Jr. Neonates were transferred within 6 hours of birth, as soon as we thought the whole litter was born, usually before the pups had nursed for the first time. There was no problem with maternal acceptance.

Vasectomies and the recovery and transfer of embryos were done under isoflurane anesthesia delivered in oxygen with a veterinary anesthesia machine. Standard aseptic surgical procedures were observed. The time of coitus was determined by recovery of vaginal plugs dropped out of wire-floored cages onto absorbent paper. Three or four days after coitus, donor uteri were flushed into Heps-buffered R1ECM culture medium maintained at 37°C for four to seven embryos were selected for normal morphology, washed, then transferred by micropipette to each horn of the pseudopregnant recipient uterus through bilateral paralumbar incisions.

Tail-cuff blood pressures in trained unheated rats (HTCG) and body weights were recorded twice a week in the rats derived from embryo transfers and from normal pregnancies born within the same 3-week period. The blood pressures of the progeny from normal pregnancies of females generated by embryo transfers were also measured to test for second-generation effects. Rats were fed Purina Noormlab chow containing 0.3% Na+ and given deionized water to drink except when 0.9% NaCl was substituted to accelerate the development of hypertension and test salt sensitivity. In others, the blood pressure was lowered by the icv infusion of 1.1 μg/h RU28318, a mineralocorticoid receptor antagonist (with vehicle containing equimolar amounts of potassium as the RU28318 solution infused icv in the controls) for 4 weeks, followed by another 4 weeks of vehicle in all rats using subcutaneous miniosmotic pumps (Alzet) as described before. All studies were terminated before or as soon as animals began to lose weight, cease to groom themselves properly, or become dehydrated, indicating renal failure and the propensity for stroke. In the RU28318 study, for example, the study ended when the controls, which had no blood pressure mutation, began to lose weight and condition.

Data were compared by analysis of variance and the Dunnett t and Fisher PLSD tests (StatView 512+, BrainPower, Inc.).

**Results**

There are environmental variables, many of which probably go unrecognized, that influence blood pressure and water-salt homeostasis. For this reason, data from rats born within 3 weeks of each other whose dams and sires were of the same age and experience were compared, rather than using data from age-matched rats over longer times. Thus the two sets of embryo transfers are treated separately. SS/Jr and SR/Jr are abbreviated to S and R, respectively, et and cf indicate embryo transferred and cross-fostered, with the donor genotype first and the recipient genotype second. All groups had at least four individuals to be included for statistical analysis, most had more than six. The combined embryo transfer success in two sets of transfers, measured as the ratio of the total number of pups weaned at 4 weeks to the total number of embryos transferred, was 27%. This figure includes embryos and neonates lost for any reason, including losses of complete litters born during several days of low humidity. When only recipients that successfully raised pups are considered, 48% of the embryo transferred were weaned. Weaned litter size varied from 2 to 8 and averaged 3.3.

S rats were, as expected, consistently larger than R rats. Typically, normally conceived S males weigh about 215 and 350 g at 8 and 12 weeks, respectively, compared to R males, which weigh 190 and 270 g at the same age. S females weigh about 180 and 240 g at 8 and 12 weeks, compared to R females, which weigh 150 and 220 g. Surprisingly, body weights at 7 weeks through adulthood were not significantly altered by litter size, cross-fostering, or embryo transfer, even considering the smaller litter size for et rats. Newborn pups were not weighed.

The blood pressures for reciprocal cross-fosternogs are shown in Fig 1. The lactational experience, including milk constituents and dam behavior, had no effect on the expected blood pressure or body weight (not shown) phenotype.

The blood pressures for two separate sets of reciprocal embryo transfers are shown in Figs 2 to 5. In the first set of transfers, Fig 2, the S male blood pressures were significantly greater (**P < 01**) than those of all other groups, including cousins and half-brothers that were transferred as embryos to R dams (SetR). There was no significant difference between the pressures of the SetR and R, RetS, RetR, or R+etS males between the ages of 6 and 12 weeks. R+etS denotes naturally conceived R pups whose dam also received and weaned two S pups as embryos. These two S rats, a male and a female, had blood pressures in the same range as the other SetR but were not included in the statistics. Substituting saline for drinking water did not affect the blood pressure of the R males but increased that of the S and SetS males at a similar rate. The slopes of the blood pressure increases after 11 weeks for the S and SetR were 6.78 and 7.79 mm Hg/week, respectively.

Fig 3 represents the blood pressures of a second group of males generated by embryo transfer. RetR blood pressures...
were left out for clarity but were not different from those for the R. The blood pressures of the S and SetS and R and RetS were typical of their strain. Pressures of 7-week-old SetR were significantly lower than those of the S and SetS (P< 01) but were not significantly different from the pressures of the R and RetS. All S blood pressures increased with age, though they were maintained on a normal salt diet. The rate of increase was similar. By 13 weeks of age, the blood pressures of the SetR had significantly diverged from those of the R but were still lower than those of the S (P< 001 for both comparisons). At week 20, all rats, including the R, received a cannula into the right lateral cerebral ventricle connected to a mini-osmotic pump delivering the mineralocorticoid receptor antagonist RU28318 or vehicle. Central mineralocorticoid inhibition predictably lowered the blood pressure in the genetically S rats and, to a much smaller extent, the R rats. Changing the pumps of all rats to deliver vehicle after 4 weeks allowed the blood pressures of the S rats, including the SetR, to increase to the level of the S rats that had not received the antagonist. The R rats’ pressures were not significantly altered.

Fig 4 represents the blood pressures of the sisters of the rats in Fig 2. Like those of their brothers, the pressures of these SetR females were not different from those of the R females and were significantly lower than those of the S (P< 01). After week 12, the SetR pressures became higher (P< 05) than those of the R females while remaining significantly lower than the blood pressures of normal S (P< 01). This increase occurred before the males were placed in the females’ cages for breeding. Blood pressures were not taken during whelping and lactation. Pressures of the SetR females had reached the level of the control S females by the time their pups were weaned. Pressures of the RetS were not different from those of the R.

Blood pressures of the sisters of the rats in Fig 3 are represented in Fig 5. Blood pressures of normally gestated S and R blood pressures were left out for clarity, but they were not different from the SetS and RetR pressures, respectively. At 7 weeks, the blood pressures of these SetS females were significantly different from those of the SetR females (P< 01) and remained so throughout normal gestation and weaning until week 21. SetR blood pressures did not become significantly greater than those of the R until week 12. As with their brothers’ group, the rates of increase in blood pressure for...
the S and SetR were similar. The blood pressure increase for the SetR accelerated at week 21, coinciding with the weaning of pups at week 20. The blood pressure of the RetR were not different from normal R blood pressures and did not change significantly over the 24 weeks of measurement.

Blood pressures of the progeny of embryo transplants before and after salt challenge were the same as those of controls of their respective strains.

**Discussion**

The cause of the hypertension in Dahl S rat is multifactoral, involving at least four to six genetic loci. An important complicating factor in reviewing the literature on the pathogenesis of hypertension in these rats is that the various inbred S and R strains are not identical, moreover, one major commercial source of inbred S rats inadvertently provided genetically contaminated S rats for several years.

Reports on the effects of alterations in dietary salt of the S dam during gestation and lactation on the adult blood pressure of the progeny vary from none to profound. Electrolyte content in R and S milk was reported to be unaltered by different levels of dietary salt, but there are conflicting reports of differing composition of S and R milk and whether genetically determined differences in milk are important for the full expression of S hypertension. Reported results from reciprocal cross-fostering, which alters the environment during the first 3 to 4 weeks after birth, are equally confusing. Cross-fostering between genetically hypertensive SHR and normotensive WKY and between inbred Dahl SS and SR rats was found to decrease the hypertension in adult SHR and SS fostered by WKY and SR, respectively, but not to alter blood pressure of WKY and SR fostered on SHR and SS. Cross-fostering between our two strains of S and R had no effect on the blood pressure, results that confirm those of Den and Rapp using slightly different Dahl SS and SR strains. Though our S and R are both very good parents and wean an average of 10+ pups, the behavior of these two strains of rats is noticeably different, the SS/jr being significantly calmer and easier to train than the SR/jr, so it was important to separate factors of the lactational environment, including nurturing factors, as well as possible differences in milk, from factors of the uterine and lactational environment that were altered by embryo transfer.

Gestation in a genetically normotensive R rat uterus significantly lowered the blood pressure in young S rats and delayed the appearance, but did not alter the progression of, hypertension or the S susceptibility to salt-induced hypertension. Gestation of the genetically normotensive R pup in a hypertensive S dam did not alter basal blood pressure or the genetic resistance to salt-induced hypertension. Blood pressure of the dam probably was not an important factor in determining the blood pressure phenotype of the offspring because the blood pressures of progeny of the SetR dams was no different from pressures of the genetically identical progeny of the S and SetS, even though the blood pressures of the SetR dams during gestation were significantly lower than those of the S and SetS dams.

Abnormalities in the hormonal environment during gestation have been implicated in hypertension in humans. The fetus is protected from high maternal glucocorticoids by placental 11β-hydroxysteroid dehydrogenases (11-HSD), enzymes that metabolize glucocorticoids. Elevated circulating glucocorticoids during gestation are associated with low birth weight, large placentas, and adult hypertension in humans and animals. It has been proposed that a relative deficiency in placental 11β-hydroxysteroid dehydrogenase activity produces a gestational hormonal milieu, notwithstanding normal circulating levels of glucocorticoids, that predisposes the adult progeny to hypertension. There is one report of 11HSD-1 deficiency in mesenteric arteries of the Dahl S rat. However, because of its very high Km, the relevance of this enzyme, rather than the 11β-HSD-2 with a much lower Km, in preventing excessive glucocorticoid action in situ is questionable.

These studies do not identify the mitigating factor(s) in the uterine environment. However, hormonal differences between the Dahl SS/jr and SR/jr have been documented that may play a role. The gene encoding the adrenal cytochrome P-450 11β-hydroxysteroid dehydrogenase (11-HSD), enzymes responsible for the biosynthesis of corticosterone (synthesized in rats instead of cortisol), 18-hydroxyDOC, and 19-norDOC, differs between the S and R, causing differences in enzyme efficiency and relative amounts of steroids produced. 19-NorDOC is a powerful mineralocorticoid that is excreted in greater amounts in the S, compared to the R, and in much greater amounts in females than in males. Urinary excretion of 18-hydroxyDOC is also significantly greater in the S rat, as is the ratio 18-hydroxyDOC/corticosterone production and excretion. Increased adrenal 18-hydroxyDOC production was reported with blood pressure in an F2 population of outbred Dahl rats. However, when inbred crosses were made, high 18-hydroxyDOC levels cosegregated with the blood pressures only in SRF × SS back-crosses. Genomic studies of 12 inbred strains of rats indicated that the more common 11β-hydroxylase is the S type and that adrenal production of 18-hydroxyDOC is not abnormally high in the SS rat but is abnormally low in the SR rat. Whether the SR type of 11β-hydroxylase has a role in lowering the blood pressure in a hypertensive environment or
in the programming of the progeny's blood pressure during gestation is not known.

There is also an increase in the production of 18-hydroxy-corticoesterone in the SS rat, however, the enzymatic basis or its relevance to hypertension is not yet known. The gene for aldosterone synthase is closely linked with that of the 11β-hydroxylase and generally cosegregates with it. Differences in the aldosterone synthase sequence and kinetics in the Dahl SS/jr and SR/jr rats may explain the lower circulating aldosterone levels in S rat compared to the R rat. While many of these differences in steroidogenesis were at first considered potential causes of high blood pressure in the S, some have now been found to be abnormal in the R rat, compared to other normotensive strains. Which, if any, of these mutations confers hypertension resistance to the R is yet unknown, but it is interesting that the R uterine environment lowered the blood pressure of the S progeny. The S uterine environment may not have altered the blood pressure of the R transfers because it was "neutral" or because the R fetus is generally resistant to a hypertensogenic environment.

The size of the lactational or gestational dam or the size of the litter had no consistent effect on progeny weight at 7 weeks of age through adulthood. While there is a positive correlation between body mass index and blood pressure in humans, the greater weight of the S compared to the R rats reflects an overall greater size. While no objective measurements were made, the relative amount of body fat of the S and R at necropsy do not appear to be different. In addition, S rats become hypertensive early, before they acquire significant amounts of body fat.

In another study, reciprocal embryo transfers between SS and SR rats were reported to have no effect on the blood pressure of the progeny. However basal blood pressures before a very high salt (8%) diet was instituted were not reported, and the success rate and numbers of animals studied were very small. In these studies, S adults from small litters were larger and survived a high-salt diet longer, even though there was no significant difference in their blood pressures compared to those of smaller S adults from large litters. We did not find changes in body weights persisting in to adulthood that correlated with litter size, nor did we allow animals to live long enough to become ill or die.

There is human and experimental evidence that "normal" blood pressure ranges for the individual is programmed centrally. This programming in the rat involves the mineralocorticoid receptor in nuclei in the area anterohventral to the third ventricle. While, from previous work, we were not surprised that the icv infusion of RU28318, a selective mineralocorticoid antagonist, lowered the blood pressure of all of the S rats, we did not expect the pressure of the SetR to be reset at the higher "normal S" level when the antagonist was withdrawn. It is unfortunate that the numbers of SetR individuals was too small to divide into two statistically defensible groups, one receiving vehicle and the other the MR antagonist icv, to check whether the blood pressure of these animals would have increased to that level without perturbation.

In summary, we have described the importance of the gestational milieu in the modulation of the genetic potential for hypertension. Which components of the fetal environment exert their effects on the programming of the blood pressure of the SS/jr remains to be defined.

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