Maternal Effects on Blood Pressure and Survivability in Inbred Dahl Salt-Sensitive Rats

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SUMMARY Maternal effects on blood pressure response to high salt diet were evaluated using inbred Dahl salt-sensitive (S/JR) and inbred Dahl salt-resistant (R/JR) rats. A cross-fostering experiment did not yield any evidence for a consistent effect of strain-specific fostering environments on the subsequent blood pressure response of S/JR or R/JR rats. A small increment in blood pressure seen only in male rats associated with the maternal R/JR environment was probably the result of effects mediated through an increment in body weight. In an experiment in which litter size was varied, weaning body weight was found to be an important predictor of survivability of S/JR rats fed a high salt (8% NaCl) diet; higher body weight was associated with longer survival in both males and females. Higher body weight at weaning also was associated with a small increment in blood pressure of S/JR rats in male, but not in female, rats. Intrauterine environment was evaluated using the embryo transplant technique. No evidence for a difference between S/JR and R/JR intrauterine environments with regard to blood pressure response of S/JR pups to salt was found. It is concluded that the early nutritional level during nursing as altered by varying litter size has important effects on body weight and survivability of S/JR rats on high salt diet, but that these effects are not mediated through changes in blood pressure. We found no evidence for genetic effects on blood pressure operating through the early maternal intrauterine or fostering environments. (Hypertension 7: 767-774, 1985)

KEY WORDS • genetic hypertension • maternal effects • salt-sensitive rats

To determine if genetically determined factors operating through the maternal environment have any effect on the subsequent developmental patterns of blood pressure and survivability in the offspring, we evaluated the fostering and intrauterine environments using inbred Dahl salt-sensitive and salt-resistant rats.

Materials and Methods

The animals used were inbred Dahl salt-sensitive and inbred Dahl salt-resistant rats produced in our laboratory from stock obtained from Dr. L. K. Dahl (Brookhaven National Laboratory, Upton, NY, USA) in 1975. Brother-sister matings had been done for 25 generations in salt-sensitive rats and 35 generations in salt-resistant rats at the time of the present experiments. The inbred strains have characteristics similar to those originally reported by Dahl for salt-sensitive (S) and salt-resistant (R) selected lines.1-3 Our fully inbred strains are designated S/JR for salt-sensitive and R/JR for salt-resistant. For the purposes of this article we will revert to calling the inbred strains S and R respectively, because, as will be seen, it is convenient to introduce a nomenclature utilizing subscripts to designate genotypes of pups as opposed to genotypes of mothers, and the use of the complete inbred strain designation becomes clumsy.

All rats were fed normal rat chow (Wayne Rodent Blox, Continental Grain Co., Chicago, IL, USA) unless noted otherwise. This diet provides 1% NaCl by weight.

Cross-fostering

In this experiment, S and R pups were cross-fostered between strains. Enough S and R breeding pairs were made to ensure that several of each kind of litter were born on the same day. All S and R mothers in this experiment had one litter previous to breeding for this experiment. They were 17 weeks old at the time of breeding. For S and R litters born on the same day, half the S pups were switched to an R mother and half the R pups were switched to an S mother. The S pups were toe clipped on the left hind-foot, the R pups on the right hind-foot, to identify the genotype of the individual pup. Cross-fostering was always done on the day of
birth, and of the eight pairs of S and R litters manipulated, in only two pairs had the pups suckled once before cross-fostering. The litter sizes after transfer of pups were 11.3 (range, 9–13) for S mothers and 11.5 (range, 9–13) for R mothers. The mothers had been fed normal rat chow (Wayne Rodent Blox) all of their lives and were continued on this diet during gestation and while nursing.

The four types of rats produced were designated as follows: \( S_s = S \) genotype pup fostered by S mother; \( S_R = S \) genotype pup fostered by R mother; \( R_s = R \) genotype pup fostered by S mother; \( R_R = R \) genotype pup fostered by R mother. With this nomenclature, the pup’s genotype is given by the first letter and the genotype on the mother fostering that pup is designated by the subscript.

The pups were weaned at 30 days of age and identified with a small numbered skin clip (National Band and Tag Co., New Port, KY, USA) placed on the back of the neck. Rats were housed four of one sex to a cage, with one of each of the four types of rats (S s, SR, Rs, R R) in each cage.

The rats were fed Wayne Rodent Blox for 2 days after weaning, and their blood pressures were taken. They were then fed a specially prepared rat chow supplemented with NaCl to yield 8% NaCl by weight (Teklad, Madison, WI, USA), and blood pressures were measured every 10 days. The day each rat died was recorded.

**Litter Size**

We wanted to manipulate the level of nutrition and thereby weaning weight of the pups. The easiest way to do this was to artificially change the litter size. The experiment used only S pups fostered by S mothers.

Sufficient S breeding pairs were made to ensure that several litters would be born on the same day. On the day of birth sufficient pups were removed from a given litter to leave five pups nursing on their natural mother. The pups removed were added to a different litter born on the same day in order to make a litter of 18 pups. Rats were weaned at 30 days of age, tagged for identification, and caged in groups of six with two to three rats originating from small litters and with three to four rats originating from large litters. The weanlings were fed regular rat chow for 2 days, and their blood pressures were measured. They were then placed on 8% NaCl diet (Teklad), and blood pressures were measured at 10-day intervals. The day each rat died was recorded. There were six litters of five rats and five litters of 18 rats. Eight rats (4 male, 4 female) were selected at random from each of the large litters and were continued in the experiment on high salt diet; all the rats available from the small litters were used.

**Embryo Transplantation**

The purpose of these experiments was to compare the effect of intrauterine environment of S and R strains on blood pressures of S rats. Thus, an S female rat bred to a S male rat provides the S embryos in the S uterine environment, but to get S embryos in the R uterine environments it was necessary to transplant S embryos to R mothers.

To perform transplants, S female rats bred to S male rats were used as embryo donors and R female rats bred to vasectomized Long-Evans hooded rats were used as pseudopregnant recipients. The S and R are both albino strains, and the black hooded coat pattern of the Long-Evans rats is dominant to albino (this fact was specifically checked in both S and R strains). Thus, any embryo accidently arising from breeding of R female rats with vasectomized hooded male rats would give rise to pups with the hooded coat pattern. No such event was seen in these experiments. Transplanted S embryos gave rise to albino pups. The experimental design is shown diagrammatically in Figure 1.

**Figure 1.** Diagram of the experimental design for embryo transplantation using S-rat embryos. Strain designations are explained in Materials and Methods.

Female rats that had been paired with male rats were checked daily for the presence of vaginal plugs in the case of R x Long-Evans pairs or for vaginal plugs or sperm or both in the case of S x S pairs. The first occurrence of either was taken to indicate Day 1 of pseudopregnancy or pregnancy respectively. Ideally, embryos were transferred from 4-day pregnant donors to 4-day pseudopregnant recipients. Occasional transplants were made between 5-day pregnant donors and 4- or 5-day pseudopregnant recipients. 

The 4-day rat embryos usually are found in the oviduct, and 5-day embryos in the uterine horns. Donor female rats were killed by cervical dislocation, and the oviducts were removed along with a small portion of the uterine horns. In the case of donors in their fifth day of pregnancy, the entire uterine horns were taken. Details of the embryo transplant procedure have been reported by Rafferty. The medium used to collect embryos was made up of one part female rat serum and two parts saline maintained at 37°C.

Recipient rats received embryos while under ether anesthesia. The uterus was approached by a mid-dorsal incision through the skin and thinner, longitudinal incisions through the musculature on each side over the uterine horns. Embryos were transferred in a minimal amount of fluid medium using a drawn out pasteur pipette and were placed directly in the uterine horns. Embryos collected from one donor were distributed
between the two uterine horns of the recipient. The time from death of the donor to transplantation into the recipient was 30 minutes.

Two types of operations were performed. In one, S embryos were actually transplanted to R pseudo-pregnant recipients and the offspring were designated S\textsubscript{R-implant} \textsuperscript{R}. In the other type of operation, pregnant S female rats underwent a sham operation during which they were subjected to the same procedure as the R recipients except that no embryos were actually transferred. The offspring were designated S\textsubscript{s.sham}. As before, the first letter designates the genotype of the pup and the subscript designates the genotype and treatment of the maternal environment (see Figure 1).

A more ideal situation might be to use S embryos transplanted to S mothers instead of S\textsubscript{s.sham} rats. As a practical matter this was not done because we wanted always to have S pups from the R and S uterine environments born at the same time so that they could be raised and studied concomitantly. Since the success rate of embryo transplant operations is low with the inbred strains (see Results) it would be virtually impossible to have successful paired transplants. Because S\textsubscript{s.sham} pups were easy to make, they were produced with sufficient frequency to ensure that they were always available when S\textsubscript{s.sham} pups were successfully produced. It was possible to have transplant and sham rats born within a few days of each other so that they were weaned at 30 days of age, tagged, and then housed in the same cages. Weanlings were fed normal rat chow for 2 days, their blood pressures were taken, and they were placed on 8% NaCl diet. Blood pressure was measured at 10-day intervals.

Embryo transplants were also done using hybrid embryos for reasons given under Results. In this case S female rats were bred to R male rats, which produced hybrid (S × R) embryos. These S × R hybrid embryos were transplanted to pseudopregnant R female recipients, or the S females pregnant with S × R embryos underwent sham operations. The offspring were designated as (S × R)\textsubscript{R-implant} \textsuperscript{R} or (S × R)\textsubscript{s.sham} respectively. Again, the nonsubscript letters designate the pup's genotype and the subscript designates the mother's genotype and treatment. This experiment is shown diagrammatically in Figure 2.

Blood pressure was measured with the rats under light ether anesthesia by the tail cuff microphonic manometer method.\textsuperscript{6} Within any given experiment, animals from the various maternal treatment types were housed in the same cages to minimize differences in postweaning environment. The operator taking blood pressures took rats at random from a given cage and did not know the identity of a rat with regard to treatment.

Data were analyzed using an Olivetti P6060 minicomputer with programs supplied by Olivetti (New York, NY, USA).

Results

Cross-fostering

Figure 3 shows the growth curves for the cross-fostered and non-cross-fostered offspring. Note that on high salt diet S rats, whether fostered by S mothers (S\textsubscript{S}) or by R mothers (S\textsubscript{R}), grew similarly to R rats for about 22 days postweaning and then their growth slowed down dramatically. This slowed growth is a consequence of their marked hypertension (see below) and sensitivity to salt toxicity. The lower body weight of S rats was significant by a 2 × 2 factorial analysis of variance in female rats at 46 days (p < 0.05) and in male rats at 33 and 46 days (p < 0.001). Figure 3 also shows that the fostering environment had a significant effect on body weight. From weaning until the end of the experiment, rats fostered by S mothers were smaller (p < 0.001) than rats fostered by R mothers. This result was from a 2 × 2 factorial analysis of variance as indicated by the asterisks in Figure 3.

Figure 4 gives the blood pressure responses for the cross-fostering experiment. The S rats (S\textsubscript{S} and S\textsubscript{R}) had significantly (p < 0.001, 2 × 2 factorial analysis of variance) higher blood pressure than R rats (R\textsubscript{R} and R\textsubscript{S}) at all points after weaning. At weaning, S and R rats were not significantly (p < 0.3) different with regard to blood pressure. In female offspring there was no effect of fostering environment on blood pressure in either S or R animals. In male offspring there was an inconsistent effect of fostering environments on blood pressure as indicated by the 2 × 2 factorial analysis of variance at each time interval. Asterisks in Figure 4 show the times at which statistical significance (p < 0.05) was found. Inspection of Figure 4 suggests this was due to a slightly higher blood pressure of S males fostered by R mothers.

Figure 5 shows the survival curves for S rats from the cross-fostering experiment. As can be seen, S rats fostered by S mothers (S\textsubscript{S}) died sooner than S rats fostered by R mothers (S\textsubscript{R}). For both male and female rats, contingency tables with rats cross-classified by fostering environment (S or R), versus alive or dead, yielded significant chi-square values (p < 0.05) for the time points from 40 to 55 days after weaning.

Litter Size

The reduced survivability of S rats fostered by S mothers (S\textsubscript{S}) was associated with the lower body weight of these rats. The cause of this decreased
survivability was not higher blood pressure in Sₘ rats as opposed to S rats fostered by R mothers (Sₘ), since either the pressures of the two groups were equal (females) or the blood pressures in the Sₘ rats were higher than those in the Sₘ rats (males). In this experiment, therefore, we wanted to manipulate body size of the rats and determine if this influenced survivability. The litter sizes of S rats were adjusted to yield litters of five and litters of 18 as given in Materials and Methods.

Figure 6 shows the body weight data for S rats from large and small litters fed 8% NaCl from weaning. Animals from small litters were significantly larger (p < 0.001, t tests) than animals from large litters at all points from weaning to the end of the experiment. The smaller rats never made up their deficit in body weight. The S rats on the 8% NaCl diet stopped gaining weight at about 30 days and then lost weight before dying. Figure 7 shows that blood pressures of S rats from small or large litters were identical for female rats, but for males the bigger rats (from litters of 5) have a moderately elevated blood pressure compared with that of the smaller animals (from litters of 18).
Cross-Fostering Experiment

FIGURE 5. Survival curves for S rats from the cross-fostering experiment. All rats were on 8% NaCl diet from weaning. The R rats all survived through the 55 days of observation and are not shown in the figure. The S rats fostered by S mothers (SS) died earlier than S rats fostered by R mothers (SR).

FIGURE 6. Body weights for rats from litters of five or 18. *Indicates the points at which the two groups differed significantly (p < 0.001) by a t test. All rats were fed 8% NaCl diet from weaning.

FIGURE 7. Blood pressure responses for S rats from litters of five or 18. The numbers in parentheses are the numbers of rats in each group at weaning. All rats were fed 8% NaCl diet from weaning. *Indicates the points at which blood pressures were statistically (p < 0.05) different by a t test.
Figure 8 shows the survival patterns for S rats from large and small litters after being fed 8% NaCl diet from weaning. The larger S rats (from litters of 5) survived longer than the smaller S rats (from litters of 18). This finding was true for both male and female rats and was shown to be significant ($p < 0.05$) by chi-square tests from days 41 to 55 after weaning for female rats and from days 40 to 46 after weaning for male rats. Chi-square tests were constructed as given above for the cross-fostering experiments.

Figures 9 and 10 show the weaning body weight plotted against days of survival postweaning on 8% NaCl diet for female and male rats respectively. The graphs include all animals for which data were available from the cross-fostering experiment, litter-size experiment, and embryo transplants given below. For both sexes body weight and survival times were significantly related ($p < 0.001$).

**Embryo Transplants**

Table 1 gives data on the success rate for embryo transplants using the protocol given in Figure 1 (see Materials and Methods) in which S embryos were employed. Although the success rate was poor, it was possible to get small numbers of transplanted rats for study. To determine whether this low success rate was due to technical difficulties, we also did transplants using hybrid embryos. Clearly, the success rate for hybrid embryos in Table 1 is much better than that for S embryos. The S embryo transplant experiments had been done as two series, one before and one after the hybrid embryo transplants. In both S embryo transplant series (which are combined in Table 1) the same low success rate was obtained. The higher success with the hybrids does not, therefore, represent increased proficiency with the technique gained by experience but probably does indicate a difference caused by the type of embryo transplanted.

Figure 11 shows the blood pressure responses to challenge with high salt diet for S female pups grown in R mothers ($S_{R\text{transplant}}$) and S female pups grown in S mothers ($S_{S\text{sham}}$). In neither the first nor the second series of experiments were there any significant differ-

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**Table 1. Data on Success Rate for Embryo Transplants**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>S</th>
<th>Hybrid</th>
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</thead>
<tbody>
<tr>
<td>Transplant operations performed</td>
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<td>12</td>
</tr>
<tr>
<td>Transplant operations producing pups</td>
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<td>6</td>
</tr>
<tr>
<td>Percent successful operations</td>
<td>12%</td>
<td>50%</td>
</tr>
<tr>
<td>Embryos transferred</td>
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<td>79</td>
</tr>
<tr>
<td>Pups born</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Percent pups to embryos transferred</td>
<td>2%</td>
<td>15%</td>
</tr>
</tbody>
</table>
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Experiment 1
(S - Females)

Experiment 2
(S - Females)

S - sham

(S - sham)

(S - transplant)

(S - transplant)

Weaning 10 20 30 Weaning 10 20 30 40

Days after weaning Days after weaning

FIGURE 11. Blood pressure response for S female rats that were either transplanted as embryos to R mothers (S - transplant) or for S female rats grown in their natural mothers (S - sham). All rats were fed 8% NaCl diet from weaning. The numbers in parentheses indicate the number of rats in each group.

ences by t tests between the S rats from the two intrauterine environments. When the blood pressure data from experiments 1 and 2 in Figure 11 were combined in a 2 x 2 factorial analysis of variance, there still was no evidence for a significant effect of intrauterine environment on blood pressure. In experiment 2, there was an unexplained 10-day delay in the rapid rise of blood pressure (see Figure 11).

Figure 11 shows data for female rats. There was only one S male pup (S - transplant) obtained from the transplants to R mothers. The blood pressure of this male pup fed high salt diet was always within 2 standard deviations of the mean of salt-fed, concomitantly raised S male pups from the S intrauterine environment (S - sham).

Figure 12 shows the blood pressure responses to challenge with high salt diet for hybrid (S x R) pups grown in R mothers ([S x R]R-transplant) and hybrid pups grown in S mothers ([S x R]S-sham). The t tests comparing blood pressures of these female hybrid pups from the S or R intrauterine environments showed no significant effects. The same was true for blood pressure responses of male pups. The data for males and females of Figure 12 were also combined in a 2 x 2 factorial analysis of variance done at each biweekly time interval. At no point did this analysis show a significant effect of intrauterine environment on blood pressure.

Discussion

Basically, no effects on blood pressure were found that could be attributed to unique differences in the maternal environment between S and R rats. This finding was true of the intrauterine environment, as evaluated by embryo transplant studies, and the fostering environment, as evaluated by cross-fostering.

There were, however, significant effects on survivability, depending on the degree of nutrition during nursing. This effect was shown in experiments in which litter size was manipulated using S rats. In large litters, a pup has access to less milk than a pup from a small litter. This fact is dramatically reflected in the weaning weight. Small pups never made up the deficit in weight and they died sooner on a high salt diet than the larger pups. The larger male pups had modest elevations in blood pressure, but in spite of their higher blood pressure (which would be expected to shorten survivability) such larger pups still outlived the smaller ones. This modest elevation in blood pressure associated with large pups was seen only in male pups.

In the cross-fostering experiments, there was a modest elevation in blood pressure seen in S males (but not females) fostered by R mothers as opposed to S males fostered by S mothers. Since the former pups also were larger and survived longer than the latter pups, the
result is similar to that seen by manipulating litter size. Thus, it is logical to conclude that $S$ mothers may have provided less total nutrition to their pups than $R$ mothers. The $S$ and $R$ mothers used in the cross-fostering experiment had already had one previous litter and were 20 weeks old at the start of the fostering period. The $S$ rats (i.e., $S/JR$) at this age fed normal rat chow invariably have severe hypertension with vascular and renal lesions. It is possible that this condition reduced their milk production, which gave rise to smaller pups.

Our results suggest that low nutrition during nursing is associated with reduced survivability of $S$ pups on 8% NaCl diet. Food restriction for rats after weaning, however, prolongs the lifespan of spontaneously hypertensive rats and rats in general when fed normal rat chow.

Using other strains of rats, some evidence for an effect of fostering environment on blood pressure has been obtained. It has been found that Sprague-Dawley rats suckled by spontaneously hypertensive mothers have higher blood pressures than Sprague-Dawley rats suckled by Sprague-Dawley foster or natural mothers. An effect of the spontaneously hypertensive fostering environment was not seen on Wistar-Kyoto rats.

No effects of intrauterine environment on blood pressure were found as evaluated by $S$ embryo transplants into $R$ mothers. This result has to be qualified because only small numbers of transplant rats could be obtained. The reasons for the low success rate of embryo transplants arise from the inbred strains themselves, since in using hybrid embryos, our transplant success rate was higher. We are inclined to attribute this result to greater vigor of the hybrid embryos.

Transplanted hybrid embryos also failed to provide evidence for intrauterine effects on subsequent blood pressure responses of the pups. The transplant experiments with hybrid embryos were done mainly as a check on the adequacy of the technical aspects of the embryo transplantations. Obviously, it is easy to produce hybrid embryos in $S$ mothers or to produce hybrid embryos in $R$ mothers merely by breeding reciprocal crosses (i.e., $S$ female $\times$ $R$ male and $R$ female $\times$ $S$ male). Reciprocal crosses between $S$ and $R$ rats also have not provided any evidence of maternal effects on blood pressure.

References
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