Rapid Communications

Genetic Contamination of Dahl SS/Jr Rats
Impact on Studies of Salt-Sensitive Hypertension

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Abstract The Dahl salt-sensitive rat (SS/Jr) is a widely used animal model of salt-sensitive hypertension. SS/Jr rats are believed to be highly inbred and uniformly sensitive to the hypertensinogenic effects of sodium chloride, but we have recently observed that SS/Jr rats from Harlan Sprague Dawley, Inc. exhibit considerable variability in their blood pressure response to supplemental dietary salt. To test the possibility that commercially available SS/Jr rats are genetically contaminated and therefore no longer fully inbred, we performed molecular genetic studies and blood pressure measurements in several groups of SS/Jr rats purchased from Harlan Sprague Dawley. We found molecular evidence of heterozygosity and/or atypical allelic variants involving loci on at least five different chromosomes. Many of the rats also failed to exhibit a salt-sensitive blood pressure phenotype. We conclude that SS/Jr rats being sold by the only commercial vendor of Dahl rats in the United States are genetically contaminated and resistant to the hypertensinogenic effects of salt. These findings raise serious questions about the interpretation of research conducted with SS/Jr rats obtained from Harlan Sprague Dawley. (Hypertension. 1994;23[part 1]:786-790.)

Key Words • rats, inbred Dahl • hypertension, sodium-dependent • genetics • sodium

The Dahl salt-sensitive rat is the most widely studied genetic model of salt-sensitive hypertension. In the 1970s John Rapp began systematic brother x sister matings of rats, and in 1985 he described the development and characteristics of fully inbred strains of Dahl salt-sensitive and Dahl salt-resistant rats.1,2 The advantages of inbred strains for biomedical research are well known, and the creation of the inbred Dahl salt-sensitive and salt-resistant strains was a major advance in the field of experimental hypertension research. To ensure that these genetically homogeneous strains would be readily available to the scientific community, Rapp provided inbred Dahl salt-sensitive (SS/Jr strain) and salt-resistant (SR/Jr strain) rats to Harlan Sprague Dawley, Inc (HSD) (Indianapolis, Ind) in 1986. Since then, multiple investigators have used the SS/Jr and SR/Jr strains for physiological, biochemical, and genetic studies of salt-sensitive hypertension.

Although commercial stocks of SS/Jr rats are purported to be inbred, we have recently observed that SS/Jr rats purchased from HSD fail to develop hypertension consistently despite being fed large amounts of dietary salt (NaCl) (unpublished observations). This is particularly surprising given that Rapp clearly demonstrated that inbred SS/Jr rats develop severe hypertension even when fed relatively modest amounts of NaCl (eg, a 0.4% NaCl diet).1 To test the possibility that commercially available SS/Jr rats are genetically contaminated and no longer fully inbred, we performed DNA typing studies on four separate groups of SS/Jr rats purchased from HSD between December 1992 and July 1993. In several shipments of rats, we also performed blood pressure studies to evaluate the salt-sensitive phenotype. In these rats, we found molecular evidence of heterozygosity and/or atypical allelic variants involving loci on at least five different chromosomes. Many of the rats also failed to exhibit a salt-sensitive blood pressure phenotype. These findings demonstrate that SS/Jr rats from the only commercial source in the United States are genetically contaminated and resistant to the hypertensinogenic effects of sodium chloride.

Methods

We studied five separate rat groups obtained from HSD between October 1992 and July 1993. In some of these groups, we measured blood pressures and performed DNA typing studies; in others, we performed only DNA typing studies. Studies were conducted on rats shipped to three separate laboratories at two different universities (University of California, San Francisco [UCSF], and the Medical College of Wisconsin, Milwaukee).

Blood Pressure Measurements

Direct measurements of arterial pressure were performed in unanesthetized, unrestrained rats fed moderate to large amounts of dietary NaCl (1% to 8% NaCl diets). Blood pressures were measured either with abdominally implanted radiotelemetry transducers connected to the lower abdominal aorta (Datasciences) or with external blood pressure transducers connected to indwelling femoral artery catheters.3,4 After surgical implantation of the radiotelemetry transducers, we allowed for 2 weeks of recovery before collecting blood pressure data for experimental analysis. In rats undergoing femoral artery pressure measurements, we allowed for a 4-
St. Lezin et al  Genetic Contamination of Dahl Rats

Fig 1. Line graph shows systolic blood pressures measured by radiotelemetry in six male Dahl salt-sensitive rats (SS/Jr) obtained from Harlan Sprague Dawley in October 1992. Rats were fed a 0.24% NaCl diet from 4 to 12 weeks of age followed by a 1% NaCl diet from 12 to 19 weeks of age. Diet potassium content was 0.5%. Radiotelemetry measurements of aortic systolic pressure were begun at 10 weeks of age. Each point represents the mean (±SD) of approximately 1000 daytime (6 AM to 6 PM) blood pressure measurements obtained in each rat during each week of the study.

5-day postoperative recovery period before collecting blood pressure data. Surgical procedures were performed in rats anesthetized by injections of 50 mg/kg IM ketamine and 2 mg/kg IM xylazine. Radiotelemetry pressures were determined from measurements continuously recorded every 5 minutes during 12-hour daytime light cycles (6 AM to 6 PM) and converted to a single mean weekly value for each rat. Femoral artery pressure measurements were determined from measurements continuously recorded for 3 hours between 8 AM and noon on 4 consecutive days. The pressures were averaged over 10-minute intervals and then converted to a single mean value for each rat. Statistical analysis was performed with ANOVA and Duncan’s multiple range test with statistical significance being defined at a value of P<.05.

DNA Typing Studies
Genomic DNA from SS/Jr rats was analyzed using polymerase chain reaction (PCR) primer pairs that amplify microsatellite repeat sequences in the rat genome (Research Genetics). From an initial screening of 25 different primer pairs from Research Genetics, we found 5 primer pairs that revealed unexpected polymorphisms: R1041, R721, R557, R371, and R513. These primer pairs were then selectively used for detailed testing of rats from different shipments. In some of the rats, we also tested for polymorphic markers in two candidate genes for hypertension, the guanylyl cyclase A/atrial natriuretic peptide receptor (GCA) and the renin gene. The GCA locus was tested using the primer pairs described by Deng and Rapp7 that amplify around microsatellite sequences in the first intron of the GCA gene. The renin gene was tested using PCR primers that amplify around a HindIII restriction site present in the fifth intron of the SR/Jr strain but not in the SS/Jr strain. The renin primers were ATGTGTCTCCTGTAGT-GACAGC (upstream) and ACTCCCTAAAAAGAAAAGCGCG (downstream). PCR was performed with the use of genomic DNA extracted from blood, liver, or spleen by standard methods.8 We typically used PCR annealing temperatures of approximately 55°C and detected the PCR products by ethidium bromide staining or autoradiography.

Results
Blood Pressure Measurements
Fig 1 shows systolic blood pressures measured by radiotelemetry in six male SS/Jr rats shipped from HSD to UCSF in October 1992. The blood pressure responses of these rats to modest amounts of dietary NaCl (≤1% NaCl) were highly variable. In four rats, systolic blood pressures exceeded 170 mm Hg, and in two others, blood pressures never exceeded 135 mm Hg. Similar patterns of variability were observed with measurements of diastolic and mean arterial pressures (data not shown).

Fig 2 shows systolic blood pressures measured by radiotelemetry in six male SS/Jr rats shipped from HSD to UCSF in March 1993. The blood pressures of these rats failed to respond to moderate amounts of dietary salt (1% to 4% NaCl). Systolic blood pressures remained below 140 mm Hg in two rats and below 160 mm Hg in four rats despite administration of a 1% NaCl diet for 6 weeks and a 4% NaCl diet for an additional 2 weeks. Similar results reflecting salt resistance were obtained for measurements of diastolic and mean arterial pressures (data not shown).

Fig 3 shows a comparison of mean arterial pressures measured through indwelling femoral catheters in three groups of female SS/Jr rats studied at the Medical College of Wisconsin: (1) a group of SS/Jr rats purchased from HSD on April 1, 1992, (2) a group of SS/Jr rats purchased from HSD between March 8 and April 6, 1993, and (3) a group of SS/Jr rats derived at the Medical College of Wisconsin from breeding stocks purchased from HSD in June 1991. All rats were maintained on a low NaCl diet until 6 weeks of age and then fed an 8% NaCl diet for 3 weeks. The blood pressures of the rats purchased from HSD in 1993 were
significantly lower than the blood pressures of those purchased in 1992 and of those bred at the Medical College of Wisconsin from stocks purchased in 1991.

DNA Microsatellite Typing Analysis

We performed DNA microsatellite typing analysis in four groups of SS/Jr rats shipped to either UCSF or the Medical College of Wisconsin in December 1992 (one shipment), March/April 1993 (two shipments), and July 1993 (one shipment). In all four shipments, we found evidence of heterozygosity and/or atypical allelic variants. PCR studies with primer pairs amplifying the R1041, R557, R721, R371, and R513 loci and the GCA locus showed evidence of genetic variability in some or all of the shipments. Overall, we found evidence of genetic heterogeneity in microsatellite loci located on chromosomes 2, 5, 6, 7, and 14. No evidence of renin gene polymorphism was found in the three HSD shipments that were tested for the HindIII site in the fifth intron of the renin gene. In SS/Jr rats bred at the Medical College of Wisconsin (n = 6), we found no evidence of polymorphism in any of the loci that showed heterozygosity or atypical allelic variants in the SS/Jr rats recently shipped from HSD. The SS/Jr rats bred at the Medical College of Wisconsin had been derived from breeding stocks purchased from HSD in June 1991.

Fig 4 shows the results of PCR studies in SS/Jr rats shipped to UCSF from HSD in July 1993 and immediately killed for DNA analysis. All three of the microsatellite markers shown in Fig 4 revealed evidence of heterogeneity among SS/Jr rats within this single shipment. Fig 5 shows an example of polymorphism at the R721 locus in SS/Jr rats shipped from HSD to the Medical College of Wisconsin between March and April 1993. Because PCR amplification of microsatellite alleles can yield more than one band, either through replication slippage or occasional false priming, it should be recognized that the presence of closely spaced doublet PCR products or multiple bands alone does not signify polymorphism. In the current examples, however, the distinctly diverse and separate band patterns between different rats are clearly indicative of genetic heterogeneity, and in some cases individual SS/Jr rats...
St. Lezin et al  Genetic Contamination of Dahl Rats

Fig 4. Polymerase chain reaction (PCR) analysis of genomic DNA of Dahl salt-sensitive rats (SS/Jr) shipped from Harlan Sprague Dawley (HSD) to the University of California, San Francisco. A, PCR products generated by primers amplifying a microsatellite repeat sequence in the guanylyl cyclase A/atrial natriuretic peptide receptor (GCA) gene on chromosome 2. Lane 1, SS/Jr rat obtained from HSD in 1990; lane 2, Dahl salt-resistant (SR/Jr) rat from HSD; lane 3, Lewis rat from HSD; lanes 4 through 9, SS/Jr rats obtained from HSD in July 1993; lane 10, DNA size marker. B, PCR products generated by amplifying the R721 microsatellite locus on chromosome 6. Lane 1, SS/Jr rat obtained from HSD in 1990; lane 2, SR/Jr rat from HSD; lane 3, Lewis rat from HSD; lanes 4 through 8, SS/Jr rats obtained from HSD in July 1993; lane 9, failed amplification; lane 10, DNA size marker. C, PCR products generated by amplifying the R1041 microsatellite locus on chromosome 14. Lane 1, SS/Jr rat obtained from HSD in 1990; lane 2, SR/Jr rat from HSD; lanes 3 through 7, SS/Jr rats obtained from HSD in July 1993.

exhibit bands that are not found in any of the other SS/Jr rats from the same shipment. Some of the alleles detected in the SS/Jr rats resembled those found in Lewis rats from HSD (Figs 4 and 5).

Discussion

The inbred SS/Jr strain was originally derived by John Rapp by repeated brother × sister matings of Dahl salt-sensitive rats.1-2 Commercially available SS/Jr rats are purported to be inbred and are presumed to be exquisitely sensitive to the hypertensinogenic effects of supplemental dietary NaCl. In the current studies, however, we have found evidence of molecular genetic and phenotypic heterogeneity in SS/Jr rats purchased from HSD, the only commercial vendor of SS/Jr rats in the United States. Specifically, in DNA typing studies in SS/Jr rats purchased from HSD by different laboratories on separate occasions, we have found evidence of heterozygosity and/or atypical allelic variants at loci on multiple chromosomes. In hemodynamic studies in SS/Jr rats purchased from HSD, we have found considerable variability in the blood pressure response to supplemental dietary NaCl; many of the SS/Jr rats we studied failed to exhibit an NaCl-sensitive phenotype.

On the basis of these findings, we conclude that the SS/Jr rats sold by HSD are genetically contaminated and do not constitute an inbred strain. The genetic heterogeneity in SS/Jr rats from HSD may be responsible for the marked variability in their blood pressure response to NaCl and in some cases complete loss of the NaCl-sensitive phenotype. Unfortunately, because of the extensive nature of the genetic contamination, it will not be possible to use these rats to determine which loci are responsible for loss of the salt-sensitive phenotype.

Excessive genetic variation within commercially available "inbred" strains of laboratory animals is a well-known problem.7 For example, genetic variation among Wistar-Kyoto (WKY) rats has been extensively documented and continues to confound attempts to compare different studies conducted with rats labeled WKY.8-10 Although genetic variation among WKY rats can be traced to the fact that breeding stocks of WKY rats were distributed long before they were fully inbred,8,11 genetic heterogeneity in SS/Jr rats cannot be attributed to premature release of noninbred breeding stocks. By the time HSD obtained the SS/Jr strain from Rapp in 1986, the rats had been brother × sister mated for 38 generations (Dr John Rapp, Medical College of Ohio, Toledo, personal communication). In inbred strains, residual heterozygosity and rare spontaneous mutations may cause a minor amount of genetic heterogeneity. In the
SS/Jr rats from HSD, however, the extent of genetic heterogeneity is far too great to be accounted for by these mechanisms.

Accidental crossbreeding of different strains can also cause genetic heterogeneity in supposedly inbred rats. The chances for accidental crossbreeding and genetic contamination of inbred strains can be minimized by comprehensive genetic monitoring programs and housing of different strains in separate buildings or rooms. Although the precise circumstances that resulted in the genetic heterogeneity in SS/Jr rats remain to be determined, we have been informed that HSD has housed SS/Jr rats in a room containing several other rat strains, including the Lewis (LEW/NHsd), Wistar-Furth (WF/NHsd), and Brown Norway (BN/BiRij/Hsd) strains (Robert Russell, HSD, personal communication). We have also been informed that Buffalo rats sold by HSD have been found to be genetically contaminated (Dr Dennis Bourdette, Oregon Health Sciences University, Portland, and Robert Russell, HSD, personal communications).

Genetic contamination of inbred strains can prove costly for investigators in both time and resources. Because of the ongoing potential for genetic contamination of inbred strains, animal breeders should continuously monitor the genetic integrity of their breeding colonies. Although commercial breeders including HSD have primarily relied on the occasional use of biochemical and immunogenetic methods to monitor the genetic integrity of their strains, such methods typically target loci that are not highly polymorphic. DNA fingerprinting techniques and PCR methods that detect highly polymorphic microsatellite markers are readily available and can provide a more comprehensive approach to the genetic monitoring of inbred strains than the older biochemical and immunogenetic methods.

The use of inbred strains in biomedical research should facilitate attempts to compare different studies both within and between laboratories. Unfortunately, the current findings of marked genetic and phenotypic variability in SS/Jr rats from HSD raise serious questions about the interpretability of studies conducted with this commercial strain. Investigators should be aware that in SS/Jr rats from HSD, variations in genotype, baseline phenotype, or the phenotypic response to an experimental maneuver may be a consequence of genetic variability in the rats bred at HSD. At this point, it appears that many if not most of the SS/Jr rats supplied by HSD are partially or even fully resistant to the hypertensinogenic effects of NaCl.

The genetic contamination of SS/Jr rats at HSD has grave consequences for hypertension research not only because it has compromised the value of studies that have been conducted with these rats but also because authentic SS/Jr rats are no longer available from a commercial vendor within North America. In preliminary studies, we have not found evidence of genetic or phenotypic heterogeneity among inbred SR/Jr rats from HSD; however, further studies should be performed to test the integrity of this strain as well. In addition to selling rats labeled as inbred SS/Jr, HSD also sells outbred Brookhaven Dahl salt-sensitive (DS) rats. The colony of DS rats is maintained by recurrent selective breeding of rats that exhibit hypertension on a high NaCl diet, and therefore, the DS rats being sold by HSD are presumably salt sensitive (or at least hypertensive). However, interpretation of studies conducted with the Brookhaven variety of Dahl salt-sensitive rats can be problematic because these rats are also genetically heterogeneous.

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