Heterogeneity of Renin Alleles in Outbred Dahl Salt-Sensitive (Brookhaven) Rats

Brian F. O'Dowd and John P. Rapp

Selectively outbred Dahl salt-sensitive (DS) and salt-resistant (DR) rats were compared with the inbred Dahl salt-sensitive (SS/Jr) and salt-resistant (SR/Jr) rats developed from the original Brookhaven stocks by J.P. Rapp. The animals were evaluated for genotype at the renin locus. The inbred strains are uniformly homozygous for their respective alleles, s in SS/Jr and r in SR/Jr. DR rats were also uniformly homozygous for the r renin allele. In DS rats, however, three renin alleles were segregating. In addition to the s and r alleles, a third allele, designated the z allele, was found. The gene frequencies in DS rats were s=0.690, r=0.284, and z=0.026. Continued use of DS and DR rats in most experimental work is inappropriate because of genetic heterogeneity in the DS stock. (Hypertension 1991;18:9-11)

Lewis Dahl at Brookhaven Laboratory, Upton, N.Y., in the early 1960s originally selected rats for sensitivity (S line) and resistance (R line) to the hypertensive effect of a high salt (NaCl) diet.1,2 The Brookhaven S and R colonies were apparently inbred for the first seven generations of selection, and then extraneous stock was introduced,3 effectively undoing any genetic homogeneity achieved during the brief initial inbreeding. The "Brookhaven colonies" are maintained by continual selection for sensitivity and resistance to salt-induced hypertension without inbreeding.3 Fully inbred strains (at least 20 generations of brother-sister matings) were, however, produced by Rapp4 from Dahl's stock starting in 1972 for R rats and 1976 for S rats. These inbred strains have been brother-sister mated for more than 50 generations as of 1990.

The noninbred Brookhaven salt-sensitive and noninbred Brookhaven salt-resistant rats are designated Dahl salt-sensitive (DS) and Dahl salt-resistant (DR), respectively. The inbred Dahl strains are designated salt-sensitive/J. Rapp (SS/Jr) and salt-resistant/J. Rapp (SR/Jr).5 All four stocks of rats are commercially available in the United States from Harlan Sprague Dawley, Indianapolis, Ind., and the inbred strains are available in Europe from Møllegaard Breeding Center, Skensved, Denmark.

Recently it was shown that SS/Jr and SR/Jr rats carried different alleles at the renin locus6,7 and that these alleles cosegregated with blood pressure in segregating populations fed a high salt diet.8,9 Alleles at the renin locus accounted for about 20% of the blood pressure difference between SS/Jr and SR/Jr strains. The purpose of the present article is to point out that noninbred DS rats are not genetically homogeneous at the renin locus, although the DR rats are.

Methods

Inbred SS/Jr and SR/Jr strains were obtained from our own colonies maintained at the Medical College of Ohio, Toledo, Ohio. Noninbred DS and DR rats (Brookhaven stocks) were obtained from Harlan Sprague Dawley.

Genomic DNA was obtained from young adult rats by standard methods from either tail biopsy tissue10 or from liver tissue11 after killing the animals with an overdose of pentobarbital. For renin genotyping genomic DNA was digested with Bgl II restriction endonuclease, the digested DNA was separated in 0.8% agarose by electrophoresis, and the DNA was transferred to nitrocellulose filters by capillary transfer.12 The filters were hybridized13 to the 2.7 kb Bgl II fragment from the first intron of the SS/Jr renin gene labeled by nick translation (kit from Boehringer Mannheim, Indianapolis, Ind.) with [32P]dCTP, and autoradiographs of the filters were prepared.13

Results

The genotype at the renin locus for 10 DR rats was determined. All rats were rr (i.e., homozygous for the...
Bgl II fragment from the first intron of hybridized to the 17 kb DNA of outbred Dahl salt-sensitive (DS/Brookhaven) rats that sz, 5, and 6; in lane 3.
in lanes 1 and 4; ss in lane
rs, z, the new renin allele observed in outbred Dahl salt-sensitive (DS/Brookhaven) rats.
renin allele found in inbred SS/Jr rats; r, the renin allele found in inbred SR/Jr rats; z, the new renin allele observed in outbred Dahl salt-sensitive (DS/Brookhaven) rats.
In contrast to DR rats, the DS rats showed a variety of genotypes at the renin locus; these genotypes and their frequencies are given in Table 1. The most common genotype was ss (46.5%) (i.e., homozygous for the s renin allele, which is the same allele present in inbred SS/Jr rats). Almost an equal number, however, were heterozygous rs (39.7%). A few were homozygous r (8.6%). In addition, a third allele was observed and is shown in Figure 1. This allele gave a Bgl II fragment of 1.3 kb and is designated (arbitrarily) as the z allele. The z allele was only seen as heterozygous sz rats (5.2%). No rz or zz genotypes were observed in the 58 DS rats that were genotyped.
The frequencies of the renin alleles in DS rats calculated from the data in Table 1 were s=0.690, r=0.284, and z=0.026. If (for simplicity) the three animals carrying the z allele are omitted, the gene frequencies in the remaining 55 animals are s=0.70 and r=0.30. Based on these gene frequencies, the expected numbers of rats in the genotypic classes are: 55(0.70)^2=26.95 for ss, 55(2×0.70×0.30)=23.1 for sr, and 55(0.30)^2=4.95 for rr. These agree almost exactly with the observed numbers of 27, 23, and 5, respectively, and of course the observed versus expected numbers of rats do not differ by a χ^2 test (p>0.99). Thus the s and r renin alleles and their related genotypes are in Hardy-Weinberg equilibrium.

**TABLE 1. Numbers and Frequencies of Rats Observed With Various Renin Genotypes Among Outbred Dahl Salt-Sensitive (DS/Brookhaven) Rats**

<table>
<thead>
<tr>
<th>Renin genotype</th>
<th>Rats (n)</th>
<th>Frequency of genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>ss</td>
<td>27</td>
<td>0.465</td>
</tr>
<tr>
<td>rr</td>
<td>23</td>
<td>0.397</td>
</tr>
<tr>
<td>sr</td>
<td>5</td>
<td>0.086</td>
</tr>
<tr>
<td>sz</td>
<td>3</td>
<td>0.052</td>
</tr>
<tr>
<td>rz</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>zz</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>58</td>
<td>1.0</td>
</tr>
</tbody>
</table>

s, The renin allele found in inbred SS/Jr rats; r, the renin allele found in inbred SR/Jr rats; z, the new renin allele observed in outbred Dahl salt-sensitive (DS/Brookhaven) rats.

**Figure 1. Autoradiograph of Southern blot of genomic DNA of outbred Dahl salt-sensitive (DS/Brookhaven) rats that was digested with Bgl II, separated in 0.8% agarose, and hybridized to the 2.7 kb Bgl II fragment from the first intron of inbred SS/Jr rats. In this system the s allele gives a band at 2.7 kb, the r allele a band at 1.5 kb, and the z allele a band at 1.3 kb. Genotypes of the rats were: rs in lanes 1 and 4; ss in lanes 2, 5, and 6; sz in lane 3.**

**Discussion**

Because DS and DR colonies are not inbred, it is known a priori that within each line, the animals are likely to be genetically heterogeneous. It has been definitively known since 1985 that DS rats did not fix the genes for high blood pressure response to salt, because seven inbred lines derived without selection from DS rats were not statistically homogeneous with regard to blood pressure response to high salt diet. Four of the seven inbred lines had very high blood pressure responses to high salt intake (205–240 mm Hg) and three of the seven inbred lines had intermediate level blood pressure responses to high salt intake (172–188 mm Hg). Such divergence of the various inbred lines derived from DS rats would have been impossible if all the genes for salt-induced high blood pressure had been fixed in the DS stock. Thus, some of the genes for blood pressure response to salt had to be segregating in the DS rats before 1985. Recent molecular genetic experiments strongly implicate s and r renin alleles as causative for blood pressure differences between the Dahl SS/Jr and SR/Jr rats. Because these alleles are shown to still be segregating in DS rats, it is obvious that the blood pressure divergence among the inbred lines derived from DS rats was likely due, at least in part, to heterogeneity at the renin locus.

The DR rats provide a counter example. There was no genetic heterogeneity in blood pressure response to salt detected in the five inbred lines derived from the DR stock. This suggests that, in fact, selection alone had resulted in fixation of alleles for low blood pressure in DR rats for the major loci controlling blood pressure response to salt. This is compatible with the fact that the appropriate low blood pressure r renin allele is fixed in DR rats.

It is worth noting that the DS stock is highly enriched for the s allele compared with the r allele, probably due to the selection pressure exerted in favor of the s (high blood pressure) allele in the early years of selecting DS rats for salt-induced high blood pressure. The rare z allele, which is still segregating in DS rats, is also interesting, but we have no explanation for its presence. We know of seven renin alleles in laboratory rats (J.P. Rapp, unpublished results). Various subsets of these alleles have been observed by others also.

A similar situation of genetic heterogeneity exists for SHR and WKY rats. It was recently shown with use of
DNA fingerprinting techniques that WKY rats are not genetically homogeneous and that the differences among commercial supplies are marked.\textsuperscript{17,18} In this case the results were also predictable since WKY rats were not fully inbred before distribution to commercial suppliers.\textsuperscript{19} Significant variability in other biological measurements in WKY rats from different commercial suppliers\textsuperscript{20,21} has also been observed.

In spite of the unquestionable superiority of inbred strains in terms of genetic purity, it is interesting to note that the availability of inbred Dahl rats starting in 1985 has had very little impact on the continued usage of the outbred Brookhaven DS and DR stocks. A review of the literature on Dahl rats from 1985 to 1990\textsuperscript{22} (omitting articles from Dr. Rapp's laboratory) revealed that the usage of the inbred Dahl rat strains constitutes about 20% of the total, and there has been no tendency at all for this to increase over the last 5 years.

The National Institutes of Health (NIH) has promulgated complex genetic guidelines for the progeny testing of DS and DR breeders by the commercial supplier. We have reviewed these guidelines. The procedure is inefficient and thus has a price. The 1991 price from Harlan Sprague Dawley is $34.25/rat for 3–4-week-old outbred DS and DR rats versus $17.95/rat for the same age inbred SS/Jr and SR/Jr rats. Thus, the majority of investigators have chosen to pay significantly more for a genetically inferior experimental animal.

The renin alleles and renin genotypes in commercially available DS rats are in Hardy-Weinberg equilibrium. One of the requirements for the Hardy-Weinberg equilibrium is that the parents of the rats studied must have been mated at random\textsuperscript{14} with respect to the renin genes. This would seem to be inconsistent with the fact that 1) alleles at the renin locus in this model have a significant effect on blood pressure,\textsuperscript{8,9} and 2) selection on blood pressure is practiced on breeder pairs by progeny testing of first litters; subsequent litters of proven breeders are the DS rats sent for experimentation. A possible explanation for this inconsistency is that the minimal testing allowed by the guidelines and followed by the commercial breeder is too weak of a selection process to cause breeding that is discernibly different than random breeding.

Although it is true that significant work has been done using DS and DR rats, it is our view that continued use of these outbred selected stocks is not justified, given that inbred SS/Jr and SR/Jr rats are available. The simple fact is that DS rats are heterogeneous at the renin locus and probably at other important loci as well. This necessarily adds undesirable variability to any experiment where DS rats are used. Moreover, the blood pressure of DS stock can be expected to vary over time due to genetic drift.

Acknowledgments

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References


Key Words: • salt-sensitive hypertension • renin • DNA fingerprinting genetics • Dahl rats
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