Use and Misuse of Control Strains for Genetically Hypertensive Rats

The article by Kurtz and Morris in this issue of Hypertension raises important issues with regard to the use of genetically hypertensive rats. These issues are 1) the origins of various stocks, 2) the definition and maintenance of the integrity of various stocks of interest in hypertension research, and 3) the construction, appropriateness, and use of control strains for comparison with hypertensive strains. The article by Kurtz and Morris traces the tortured origin of the Wistar-Kyoto (WKY) "strain" as a control for spontaneously hypertensive rats (SHR); clearly, the need for a control strain here was an afterthought and not all WKY are likely to be genetically identical. Their article illustrates the frustration of leaving unanswered the question of how to obtain genetic information from a hypertensive strain.

The problems relating to control strains originate, in my view, from the unrealistic and inappropriate expectations imposed on a comparison of a hypertensive and control strain rather than from the undefined origins and possible genetic inappropriateness of controls. It is obvious from the literature on SHR that many investigators compare SHR and WKY for some biochemical or physiological trait (call it "trait X") and then conclude that the strain difference in trait X may be causally related to blood pressure differences. The problem is that, with this kind of information, the strain difference in trait X may be the result (rather than the cause) of strain differences in blood pressure, or strain differences in trait X may be due to genetic drift. Genetic drift is the chance selection and fixation of the contrasting genes controlling trait X in the two different strains. Strain differences arising from genetic drift have nothing to do with strain differences in blood pressure.

If trait X differs between a hypertensive and a normotensive control strain the appropriate conclusion is simply that there are strain differences in trait X and there are strain differences in blood pressure. Why make such comparisons if this is all that can be concluded? Obviously, one has to determine if strain differences exist in a trait of interest as a first step in determining if there is any difference worthy of further study. To unravel the cause and effect relationship between trait X and blood pressure, however, one must be willing to do more than make strain comparisons.

The meaning of strain-differences in trait X vis-à-vis strain differences in blood pressure can be found by determining if trait X and blood pressure are genetically separable or inseparable. That is, one tests in breeding experiments whether the association of trait X and blood pressure (i.e., a component of blood pressure) is retained in genetically segregating populations (i.e., populations in which genes can recombine at random). If the gene (or genes) controlling trait X is one of the genes influencing blood pressure, then it will remain associated with an increment of blood pressure in genetically segregating populations. If the gene (or genes) controlling trait X is not one of the genes influencing blood pressure, then obviously when genes are...
allowed to mix randomly with regard to one another any association of trait X and blood pressure will be lost. Actually, this genetic testing must be done with some understanding of the genetic principles involved because the conclusions possible from such experiments are dependent on the type of inheritance (monogenic or polygenic) of trait X. A paradigm for doing such experiments has been given previously. The method has been applied successfully to determining the cause and effect relationships between blood pressure and such traits as arylesterase isoenzymes,1 steroid profile,4 vascular responsiveness,5-8 behavioral patterns,6 red blood cell membrane ion transport,10 isoelectric forms of renin,11 and sodium balance.12

In the application of this genetic paradigm,2 the strains crossed to generate a segregating population are selected for technical reasons as the ones with the greatest strain differences in trait X and blood pressure. For example, in studying a single genetic locus influencing vascular reactivity that did cosegregate with an increment in blood pressure, SHR were crossed with inbred Dahl salt-resistant rats (SR/Jr).3 There was no requirement to use WKY in these genetic experiments. Such statements should not be misunderstood to mean that a control strain genetically related to a hypertensive strain is not desirable for each hypertensive strain. If one is only going to make strain comparisons, then a control genetically related to the hypertensive strain is desirable. Although only limited interpretation can be made from such strain comparisons, it is the best that can be done under the constraint of performing no further tests.

An illustration of the limitations of strain comparisons is in order. A comparison of adrenal renin in SHR, WKY, SR/Jr, and inbred Dahl salt-sensitive rats (SS/Jr) is summarized in Table 1. What conclusions might be drawn from Table 1? Comparison of SHR and WKY leads to the conclusion that high adrenal renin and high blood pressure are associated, whereas comparison of SS/Jr and SR/Jr leads to exactly the opposite conclusion (i.e., that low adrenal renin and high blood pressure are associated). One might also logically conclude that adrenal renin and blood pressure are unrelated because, for example, SHR and SS/Jr both have high blood pressure but different adrenal renin levels or SHR and SR/Jr both have high adrenal renin levels but different blood pressures. Actually, none of these conclusions are valid for reasons relating to the polygenic nature of blood pressure (to be discussed later). Only a genetic analysis of adrenal renin and blood pressure in the same segregating population can answer the question of whether there is a genetic association of adrenal renin and blood pressure, and the same genetic analysis may, or may not, provide insight into cause and effect relationships between the two traits if they are genetically inseparable.

Such strain comparisons are spurious because blood pressure is influenced by many genetic loci. Since the additive effects of these genes determine the characteristic blood pressure of a strain, strain comparisons are confounded because we are only able to determine the net additive effect of the genes (i.e., the final strain blood pressure) and this provides no information about the similarities or differences between strains for the components causing the blood pressure differences. For example, suppose a gene (or genes) that caused high adrenal renin actually did cause an increment in blood pressure, thus being a component of the type of hypertension seen in SHR. SS/Jr could still be hypertensive even though they carry the gene (or genes) for low adrenal renin and a decrement in blood pressure; genes for increased blood pressure at other loci might be present in SS/Jr, and the net additive effect over all loci would still be high blood pressure. A theoretical numerical example of how this works is given in Reference 2.

Basically, it is impossible to construct an ideal control strain for comparison with a hypertensive strain. An ideal control strain would be genetically identical to the hypertensive strain, except that at the genetic loci influencing blood pressure the control would carry a contrasting allele for low blood pressure. In this theoretical case

<table>
<thead>
<tr>
<th>Strain</th>
<th>Adrenal renin</th>
<th>Blood pressure</th>
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<tbody>
<tr>
<td>SHR</td>
<td>High</td>
<td>High</td>
<td>Naruse and Inagami13</td>
</tr>
<tr>
<td>WKY</td>
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<tr>
<td>SR/Jr</td>
<td>High</td>
<td>Low</td>
<td>Baba et al.14</td>
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biochemical or physiological differences between strains found in young rats before
the development of strain differences in blood pressure would be likely causes of
hypertension. (Biochemical or physiological strain differences found after strain dif-
ferences in blood pressure developed could be, depending on their nature, conse-
quences of blood pressure differences.) Obviously, selective inbreeding for high and
low blood pressure from a foundation stock does not yield this ideal situation because
any genetic loci that are polymorphic in the base population may have contrasting
alleles fixed at random in one strain or the other, thus creating strain differences that
have nothing to do with blood pressure. This is apparently what has occurred for
certain behavioral traits that differ between SHR and WKY, since these traits were
shown to be unrelated to blood pressure by genetic analysis.9

Another alternative is to construct congenic strains that might approach the theoreti-
cal ideal but will still have shortcomings. Construction of congenic strains is fairly
straightforward for single, well-defined mendelian loci,15 but it is not obvious how
well the technique can work for a polygenic trait like blood pressure. In the single locus
case where one wants to move a given allele from strain A to strain B, strains A and B
are crossed to produce F1 rats. The F1 rats are then backcrossed to B (i.e., F1 X B
breeding), and the offspring are screened for the genotype at the locus of interest. Half
of the backcross population will be heterozygotes (i.e., they will carry one strain A
allele and one strain B allele), and half will be homozygotes (i.e., they will carry two
strain B alleles) at the locus of interest. Heterozygotes are selected on the basis of
phenotypic testing, another backcross to strain B is made, and, again, heterozygotes
for the locus of interest are selected. This procedure is repeated for seven cycles, at
which point more than 99% of the loci in the seventh backcross population are
homozygous for the B allele.18 Since heterozygotes for the locus of interest are always
selected, the desired allele from strain A is maintained in the backcross populations,
but the rats will eventually become homozygous for the strain B alleles at (almost) all
other loci. After many repeated backcrosses one has only to breed two rats heterozy-
gous at the locus of interest and select rats homozygous for the A strain allele at this
locus. Such homozygotes will be (almost) identical to the original strain B rats, except
that they will have the strain A allele at the locus on which selection was made. A good
example is the transfer of a gene for diabetes from one mouse strain to another for the
purpose of studying the effect of genetic background on the diabetes gene.16

Actually, one does not transfer only the gene of interest from strain A to B in the
preceding example; genes linked to the gene of interest are transferred as well (the
greater the number of backcrosses, the smaller the number of unwanted linked genes
transferred).13 This means that the congenic strains may differ at a modest, undefined
number of linked loci besides the locus on which selection was made during back-
crossing.

Although production of congenic strains is straightforward for well-defined genetic
polymorphisms (mendelian traits), the idea of using repeated backcrossing to produce
a control congenic strain for a hypertensive strain presents some interesting challenges.
Suppose, for example, one wishes to make a “better” control than WKY for SHR. A
SHR X WKY cross is made, and the F1 offspring are backcrossed to SHR. From this
backcross population one has to select breeders (for the next backcross to SHR) that are
heterozygous at all loci influencing blood pressure. How is this to be done? Blood
pressure can be measured, and the rats with the lowest pressures selected for breeding.
In such work, ultimately, one individual rat from this backcross population must be
used for the next cycle of backcrossing. Given that one is selecting for a polygenic trait
like blood pressure, there is no way to be sure at each cycle of backcrossing that the
individual rat selected is heterozygous for all the loci influencing blood pressure.
If the individual rat is homozygous (it can only be homozygous for the allele
from SHR) at a given locus controlling blood pressure, then the allele from WKY (for
low blood pressure) is irretrievably lost from the breeding program. Therefore, the
unwanted allele from SHR, not the desired allele from WKY, will appear at this locus
in the final congenic strain. If there are many genetic loci influencing blood pressure,
each with modest incremental effects, then the congenic control strain may end up
being very similar or even identical to the SHR. If, on the other hand, as does seem
possible in SHR,17 there is one single locus with a strong effect on blood pressure, then
backcrossing to the hypertensive strain with counterselection for low blood pressure
might produce a congenic control of some use.

Actually, such backcrossing experiments with selection on blood pressure have
been performed twice with SHR. Tanase successfully transferred the allele from SHR to a major locus influencing blood pressure to normotensive inbred Donryu rats, and in a separate experiment, the Donryu allele at this major locus was successfully transferred to SHR. The fate of these congenic strains since 1979 is obscure. Judy et al. made backcrosses to normotensive inbred Wistar-Lewis rats with selection for high blood pressure in an attempt to transfer the alleles for high blood pressure from SHR to the inbred Wistar-Lewis strain. At least in terms of producing a useful congenic strain, the results were ambiguous and a definitive congenic strain was never produced.

In summary, the comparison of hypertensive and normotensive strains of rats for differences in biochemical or physiological traits is a necessary first step in finding the causes of genetic hypertension. The limitations of such comparisons are unsolvable without further genetic analysis of the relationship of blood pressure to each trait. A misunderstanding of the true nature of such strain comparisons has given rise to the false expectation that an ideal control strain should or can be developed for each hypertensive strain and to the continued propagation of unsustainable inferences about the causes or possible causes of genetic hypertension from strain comparisons per se.

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References

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