Biological Variability in Wistar-Kyoto Rats
Implications for Research with the Spontaneously Hypertensive Rat

THEODORE W. KURTZ AND R. CURTIS MORRIS, JR.

SUMMARY The spontaneously hypertensive rat (SHR) initially bred in Kyoto is the most widely studied animal model of essential hypertension. As controls for the SHR, most workers have used normotensive descendants of Wistar rats from the colony in Kyoto from which the SHR strain was derived (Wistar-Kyoto rats, WKY). But the presumption that WKY are serviceable controls for SHR rests on the tacit assumption that all WKY constitute a single inbred strain. It appears, however, that whereas the National Institutes of Health distributed breeding stocks of SHR after they had been fully inbred (i.e., after 20 generations of brother-sister mating), the breeding stocks of WKY were distributed before they had been fully inbred. Accordingly, the biological variability of WKY may be greater than that of SHR. To investigate this possibility, we obtained SHR and WKY from two of the largest commercial suppliers in the United States and systematically measured the growth rate and blood pressure of these rats under identical physical and metabolic conditions. We found that WKY from one source differed from those of the other in both growth rate and blood pressure. In contrast, the SHR from the two suppliers were not different with respect to either growth rate or blood pressure. Because the National Institutes of Health may have distributed breeding stocks of WKY as early as the F6 generation, it is possible that rats currently designated as WKY do not constitute a single inbred strain. Thus, interpretation of studies employing “the Wistar-Kyoto rat strain” as a control for the SHR may be much more problematic than has previously been recognized.

(KEY WORDS • hypertension • genetics • Wistar-Kyoto rats • spontaneously hypertensive rats • inbred strains

In 1963, Okamoto and Aoki1 reported from Kyoto, Japan, that they had selectively bred Wistar rats to be spontaneously hypertensive. Established as an inbred strain in 1969 at the National Institutes of Health (NIH), the spontaneously hypertensive rat (SHR) remains the most widely studied animal model of essential hypertension (i.e., persistent high blood pressure of unknown causation).2 As controls for the SHR, most workers have employed normotensive descendants of Wistar rats that NIH investigators obtained in 1971 from the colony in Kyoto from which the SHR strain was originally derived (Wistar-Kyoto rats, WKY). Because SHR and WKY were not developed as congenic strains, WKY are manifestly less than ideal controls for SHR.2 The presumption that WKY are serviceable controls for SHR rests on the tacit assumption that all WKY constitute a single inbred strain.3 4 It appears, however, that whereas the NIH distributed breeding stocks of SHR after they had been fully inbred (i.e., after 20 generations of brother-sister mating), the breeding stocks of WKY were distributed before they had been fully inbred (advertising material, Taconic Farms, Germantown, NY, USA, 1985; personal communication, Sandra J. Sgrulloni, Charles River Laboratories, Wilmington, MA, USA, 1985; price list, Charles River Laboratories, 1985).5 Accordingly, the biological variability of WKY may be greater than that of SHR. To investigate this possibility, we obtained SHR and WKY from two of the largest commercial suppliers in the United States and systematically measured the growth rate and blood pressure of these rats under identical physical and metabolic conditions. We found that WKY from one source differed from those of the other with respect to...
both growth rate and blood pressure. In contrast, the SHR from the two suppliers were not different with respect to either growth rate or blood pressure.

**Materials and Methods**

Weanling, 4-week-old male SHR and WKY were received simultaneously from Charles River Laboratories, Inc. (Wilmington, MA, USA) and Taconic Farms (Germantown, NY, USA). All animals originated from stocks that had been cesarean-derived at one point and then continuously maintained behind special barriers to prevent infection by a variety of specific murine pathogens. Charles River Laboratories and Taconic Farms received their original breeding stocks of SHR and WKY from the NTH between 1972 and 1974 (price list, Charles River Laboratories, 1985; advertising material, Taconic Farms, 1985). When the current studies were performed, the only other commercial supplier of SHR and WKY in the United States, Harlan Sprague-Dawley, was marketing SHR and WKY that were not cesarean-derived and virus antibody-free. Therefore, we did not include SHR and WKY from Harlan Sprague-Dawley in our studies.

Immediately upon arrival in our laboratory, all rats (nine per strain per vendor) were housed three to a cage and maintained on a standard rat diet and tap water ad libitum in the same room, with a 12-hour light/dark cycle throughout the experiment. Thus, all rats were studied under the same environmental conditions and in the same way. Body weights were measured in all rats once each week by the same technician using a triple-beam balance. After 16 weeks, mean arterial pressures were measured directly in the unanesthetized, unrestrained state through indwelling femoral arterial catheters as previously described.\(^6\)\(^7\) On two additional occasions, the body weight study was repeated using newly received shipments of rats from Charles River Laboratories and Taconic Farms. All procedures were performed in accordance with the guidelines of the Committee on Animal Research of the University of California, San Francisco.

The slopes of the body weight curves were compared using analysis of covariance. The mean body weights of the rats from Taconic Farms were compared with those from Charles River Laboratories using Scheffe’s test. Statistical significance was defined as a \(p\) level below 0.017 after the Bonferroni correction for multiple comparisons (0.05/3) because the body weight studies were repeated on three separate occasions. No differences in blood pressure were detected between SHR from the two suppliers (see Figure 1).

**Results**

As judged by the slopes of the body weight curves, the growth rate of WKY from Taconic Farms was significantly greater than that of WKY from Charles River Laboratories (Figure 1). The mean body weight of WKY from Taconic Farms became significantly greater than that of WKY from Charles River Laboratories within 1 week of starting the experiment. The results of the repeat body weight studies confirmed the finding of a substantial difference in growth rate between WKY from the two suppliers (see Figure 1). The growth rate of SHR from Taconic Farms was not significantly different from that of SHR from Charles River Laboratories (Figure 2).

In the WKY from Charles River Laboratories, mean arterial pressure was significantly greater than that in WKY from Taconic Farms (Figure 3). We detected no differences in blood pressure between SHR from the two suppliers (see Figure 3).

**Discussion**

The current findings demonstrate that WKY from Charles River Laboratories differ from WKY from Taconic Farms with respect to both growth rate and blood pressure, whereas no differences were detected in either growth rate or blood pressure between the SHR from the two sources. From study to study, certain biological differences between SHR and WKY appear to vary greatly in magnitude and some even in direction (Table 1). Although such conflicting results might reflect differences in experimental design and technique among different investigators, the current findings raise the possibility that some of these variable results could reflect inherent biological variability.
BIOLOGICAL VARIABILITY IN WISTAR-KYOTO RATS/Kurtz and Morris

Table 1. Study-to-Study Variability of 10 Physiological or Metabolic Characteristics of SHR Relative to Those of WKY

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Reference number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>SHR&gt;WKY 9</td>
</tr>
<tr>
<td></td>
<td>SHR=WKY 10</td>
</tr>
<tr>
<td></td>
<td>SHR&lt;WKY 10</td>
</tr>
<tr>
<td>Neonatal blood pressure</td>
<td>11 12 13</td>
</tr>
<tr>
<td>Plasma volume</td>
<td>14 15 16</td>
</tr>
<tr>
<td>Plasma renin activity</td>
<td>17 18 5</td>
</tr>
<tr>
<td>Intestinal transport of calcium</td>
<td>19 20 8</td>
</tr>
<tr>
<td>Plasma calcitriol</td>
<td>— 8 21</td>
</tr>
<tr>
<td>Plasma calcidiol</td>
<td>8 21</td>
</tr>
<tr>
<td>Plasma phosphorus</td>
<td>— 9 22</td>
</tr>
<tr>
<td>Portal vein contractility</td>
<td>23 24</td>
</tr>
<tr>
<td>Erythrocyte Ca^{2+}, Mg^{2+}-ATPase activity</td>
<td>25 26</td>
</tr>
</tbody>
</table>

*This listing is intended to be representative, not comprehensive.

in the WKY themselves. These observations raise serious questions about the interpretability of studies employing “the Wistar-Kyoto rat strain” as a control for the SHR.

In 1978, Sinaiko and Mirkin reported differences between WKY from two suppliers with respect to basal

and isoproterenol-induced renin release from in situ perfused kidney preparations. However, their study was not originally designed to compare WKY from different sources. Before these workers were able to complete their originally intended comparison of SHR and WKY, the first supplier of WKY discontinued their breeding. The authors subsequently noted that WKY from a new source, Taconic Farms, were different from those of the original source, Biolab. Simultaneous comparison of WKY from the new source with those from the original source does not appear to have been made.

In 1976, Mullins and Bank reported differences between SHR from two different colonies with respect to the urinary excretion of sodium after volume expansion with isotonic saline. However, SHR from the colony studied by Mullins and Bank are not commercially available; some were obtained from a colony maintained by a drug company and others from a commercial source that is no longer in the business of selling SHR.

The currently observed differences in growth rate and blood pressure between WKY from different sources might be related to factors such as spontaneous mutation, genetic contamination of the breeding stocks, or nongenetic influences such as vertically transmitted disease or differences in the prenatal and neonatal environments existing at the two breeding facilities. However, in light of what we have also found about the developmental history of WKY, strong consideration must be given to the possibility that the observed phenotypic variability in WKY is due, at least in part, to residual heterozygosity of the breeder WKY originally distributed by the NIH. In 1971, the NIH received “noninbred” normotensive Wistar rats from the Kyoto colony and started a program of systematic inbreeding. In 1974, when these
rats were only at F_{10} and F_{11} generations, the NIH distributed breeding pairs to Charles River Laboratories (personal communication, Sandra J. Sgrulloni, Charles River Laboratories, 1985) and to Taconic Farms (advertising material, Taconic Farms, 1985). It appears that NIH distributed some breeder WKY as early as F_{1}.

Rats separated before F_{9} might be considered to belong to different strains. These circumstances almost ensure that WKY obtained from different vendors are genetically different.

In the article that describes the initial development of the SHR, Okamoto and Aoki stated that the rats were selected from a Wistar strain that had been maintained by inbreeding. Therefore, it is conceivable that the normotensive Wistar rats sent from Kyoto to NIH in 1971 were at least partially inbred. However, the precise circumstances of the brother-sister mating are not clear because records from the NIH indicate that 1) the SHR were developed from an "outbred Wistar-Kyoto male" and 2) the Wistar rats from Kyoto used by the NIH to breed WKY were from "noninbred" stock. Furthermore, the report of the Committee on Care and Use of Spontaneously Hypertensive Rats published in 1976 states that genes "are still segregating in the WKY rat strain."

After receiving stocks of WKY from the NIH, Charles River Laboratories continued to brother-sister mate the rats but Taconic Farms did not (price list, Charles River Laboratories, 1985; advertising material, Taconic Farms, 1985). Some research laboratories also established breeding programs with SHR and WKY, but the number and nature of such programs are unknown. Although certain facilities may have continued to brother-sister mate the partially inbred stocks of WKY, this does not mitigate the potential problem of genetic variability among so-called WKY from different sources; such facilities may now have substantially different inbred substrains, or even different strains, although each is designated as WKY.

In the current study, we did not detect any differences in blood pressure or body weight between SHR from different suppliers. Charles River Laboratories and Taconic Farms obtained their original breeding stocks of SHR from the NIH at generations F_{32} and F_{33}, respectively. The one other large commercial supplier of SHR rats in the United States, Harlan Sprague-Dawley, received breeding stocks of SHR from the NIH 10 years after the rats had been fully inbred. We do not mean to imply, however, that SHR from different sources are genetically identical. Indeed, Kunz and Gill have found that different populations of SHR can have different RT8 erythrocyte antigens. But, in commercially available rats designated as SHR in the United States, any strain variability that does exist cannot be related to the distribution of noninbred breeding stocks. Okamoto et al. systematically inbred a substrain of SHR to be stroke-prone, but these rats are clearly distinct from standard SHR and have been given a specific designation (stroke-prone SHR, SHRSP).

The development of the SHR is an important milestone in the history of hypertension research. The initially perceived potential of the SHR for investigating pathogenetic mechanisms of essential hypertension may have prompted a sense of urgent need for a genetically related normotensive control, and this may account for the distribution of WKY before they were fully inbred. Although recognizing that the ideal control would have been a strain of rats identical to the SHR except for the genes for increased blood pressure, the Committee on Care and Use of Spontaneously Hypertensive Rats of the National Research Council stated: "Since we do not operate in an ideal world, the next best approach probably is to use as a control the WKY rat strain, the base normotensive stock from which the SHR rat was derived." However, the present findings demonstrate important biological variability in WKY from different sources and suggest that rats currently designated as WKY might not constitute a single inbred strain. Thus, interpretation of studies employing the "Wistar-Kyoto rat strain" as a control for the SHR may be much more problematic than has previously been recognized.

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References

In view of the very provocative and interesting paper of Drs. Kurtz and Morris, the editors invited Dr. Yukio Yamori to write a brief commentary on the genealogical backgrounds of the Wistar-Kyoto rat and the spontaneously hypertensive rat. The following is Dr. Yamori's comment.

Commentary

YUKIO YAMORI

In their introduction, Kurtz and Morris stated that the inbred strain of SHR was established at NIH in 1969. The inbred strain was, in fact, first established in 1963 by our hands at Kyoto University, and Dr. Carl Hansen independently established the inbred strain of SHR at NIH. The first colony of SHR from Kyoto University was introduced to NIH at the F13 generation in 1967. When I was a research associate at NIH, response to urgent requests from researchers.

Since these two strains are being used extensively throughout the world, researchers should be aware of the genealogical background of SHR and WKY. We also noted recently that genetic markers of WKY, such as asylosterase isozyme patterns, differed among the available strains of WKY (unpublished observation). Such information is useful for researchers who are using SHR in comparison with WKY and may assist in understanding the correct usage of SHR as well as the control WKY strain.

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