Myocardial hypertrophy of normotensive Wistar-Kyoto rats

Ernesto A. Aiello, María C. Villa-Abrille, Eduardo M. Escudero, Enrique L. Portiansky, Néstor G. Pérez, María C. Camilión de Hurtado, and Horacio E. Cingolani. Myocardial hypertrophy of normotensive Wistar-Kyoto rats. Am J Physiol Heart Circ Physiol 286: H1229–H1235, 2004. First published November 20, 2003; 10.1152/ajpheart.00779.2003.—In our studies with spontaneously hypertensive (SHR), Wistar-Kyoto (WKY), and Wistar rats, we observed normotensive WKY rats with cardiac hypertrophy determined by a greater left ventricular (LV) mass (LVM)-to-body weight (BW) ratio (LVM/BW) than that of normotensive Wistar rats. Thus we compared the following parameters in SHR, WKY, and Wistar rats: LVM/BW, cell capacitance as index of total surface area of the myocytes, length, width, and cross-sectional area of cardiac myocytes, LV collagen volume fraction, and myocardial stiffness. The LVM/BW of WKY (2.41 ± 0.03 mg/g, n = 41) was intermediate between SHR (2.82 ± 0.04 mg/g, n = 47) and Wistar rats (1.98 ± 0.04 mg/g, n = 28). A positive correlation between blood pressure and LVM was found in SHR, whereas no such relationship was observed in WKY or Wistar rats. Cell capacitance and cross-sectional area were not significantly different in SHR and WKY rats; these values were significantly higher than those of Wistar rats. The cell length was smaller but the width was similar in WKY compared with SHR. Papillary muscles isolated from the LV of WKY and SHR were stiffer than those from Wistar rats. Consistently, a greater level of myocardial fibrosis was detected in WKY and SHR compared with Wistar rats. These findings demonstrate blood pressure-independent cardiac hypertrophy in normotensive WKY rats. Collagen; myocytes; hypertension; cardiac mass; stiffness

The association between hypertension and cardiac hypertrophy has been widely studied and accepted. However, the correlation between blood pressure (BP) and cardiac hypertrophy is not high (10, 16), even when BP was monitored by ambulatory measurements (16). Moreover, not all hypertensive patients develop cardiac hypertrophy (40) and, indeed, in some patients, the development of hypertrophy can precede the emergence of hypertension (10) or has no obvious cardiovascular cause (24). Epidemiological evidence indicates that cardiac hypertrophy constitutes an independent risk of cardiac mortality and morbidity, regardless the etiology (26). Experimental evidence also indicates that left ventricular (LV) hypertrophy can develop independently of BP (7, 16, 20, 23). The expression of certain spontaneously hypertensive rat (SHR) genes contributes to LV hypertrophy independently of BP (7, 20, 23).

Sebkhi et al. (37) reported that the LV mass (LVM) of normotensive Wistar-Kyoto (WKY) rats was greater than that of another normotensive strain (Fischer-344), despite the fact that the latter group of rats presented significantly higher values of mean arterial pressure than the former one. Masciotra et al. (29) have recently reported that normotensive WKHA rats, derived from crosses between WKY and SHR, exhibited larger LVM than WKY rats, indicating that the expression of certain genes may increase this mass even in the absence of elevated BP. In posterior studies, the same group of investigators more recently proposed that these recombinant inbred WKHA rats could represent a good model for pressure overload-independent LV pressure (11). In this study, we report that the widely used normotensive control for SHR, the WKY rat strain, in fact, develops pressure overload-independent myocardial hypertrophy, with values of cell size, fibrosis, and diastolic dysfunction of a magnitude close to those of the hypertensive strain.

Materials and Methods

Experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996) with male age-matched Wistar, WKY, and SHR (4-5 mo old). The three rats strains were inbred in our own animal facility. The SHR and WKY rats were originally derived from Charles River Breeding Farms (Wilmington, MA). Systolic blood pressure was recorded by the tail-cuff method (5). Animals were euthanized under ether anesthesia, and their hearts were removed. The LV with the septum and the right ventricle were separately weighed and normalized by body weight to determine cardiac hypertrophy.

Echocardiographic examination. Rats were monitored echocardiographically under light anesthesia (35 mg/kg ip pentobarbital sodium). Cardiac geometry and function were evaluated with a two-dimensional M-mode echocardiogram with a 7-MHz linear transducer. All measurements, including LV wall thickness systolic and diastolic dimensions were performed according to the American Society of Echocardiography leading-edge method (34). LVM was calculated as previously described (27). LV wall thickness was calculated in each rat as the mean of the end diastolic thickness of the septum and the end diastolic thickness of the posterior wall.

Stiffness. Myocardial stiffness was determined in LV papillary muscles as previously described (5). After being mounted, the unstretched length of each muscle (Lo), thickness, and width were determined with a stereomicroscope (SZ30 Olympus Zoom) set at a total magnification of ×30. The muscles were progressively stretched to the length at which they developed the maximal twitch force (Lmax) in steps of 10% of Lo. The force was normalized to the cross-sectional area of each muscle, which was calculated multiplying thickness by width and corrected by a factor of 0.75, assuming the shape of an ellipse. The stress-strain relationship of each muscle was fitted to an exponential equation. From each fitting, the stress values at given values of strain were estimated by interpolation and plotted as a function of strain.

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Address for reprint requests and other correspondence: E. A. Aiello, Centro de Investigaciones Cardiovasculares, Facultad de Ciencias Médicas, 60 y 120, La Plata 1900, Argentina (E-mail: aaiello@atlas.med.unlp.edu.ar).
Morphometry. Ventricular tissue was fixed in buffered 10% formaldehyde and paraffin embedded. LV coronal sections (5 μm thick) at the equator were stained for determining cardiomyocyte cross-sectional area (CSA) and quantifying collagen volume fraction (CVF) as previously described (5). To assess CSA, only round to ovoid cells with visible round nucleus were considered, and 50 cells were counted in at least 10 images obtained from each left ventricle. CVF was calculated as the sum of all connective tissue areas divided by the total section surface, in no <10 images. Perivascular collagen was excluded from this measurement.

Patch-clamp recordings of membrane capacitance. Rat cardiac myocytes were isolated according to the technique previously described (1). LV myocytes were diluted and placed in a perfusion chamber and superfused with bath solution at a flow rate of 1.5 ml/min. The standard whole cell configuration of the patch-clamp technique was used for voltage-clamp recordings with a patch-clamp amplifier (Axopatch 200A, Axon Instruments; Foster City, CA). Capacitative currents evoked by a step depolarizations to −55 mV (10 ms) from a holding potential of −75 mV were recorded to determine cell capacitance: The external solution contained (in mM) 133 NaCl, 5 KCl, 1.2 MgSO4, 0.8 MgCl2, 10 glucose, 1 CaCl2, and 10 HEPES, pH 7.35, with 5 mmol/L NaOH. The pipette solution contained (in mM) 130 K+ gluconate, 10 KCl, 5 Na2ATP, 0.5 MgCl2, 1 EGTA, and 10 HEPES, pH 7.15 with KOH.

Cell size. Myocytes bathed in external solution were monitored with a charge-coupled device video camera and width and length of the myocytes were measured with a cell edge detector (Crescent Electronics; Sandy, UT).

Statistics. Data are presented as means ± SE. One-way ANOVA, followed by the Student-Newman-Keuls post hoc test, was used to assess differences between groups with the significance level set at \( P < 0.05 \).

RESULTS

Figure 1 shows a plot of LVM assessed by the postmortem weight of the isolated hearts, as a function of BP, for the three strains of rats used in this study, Wistar, WKY, and SHR. LVM of WKY (7.36 ± 13 mg, \( n = 41 \)) was lower than that of SHR (887 ± 16 mg, \( n = 47 \), \( P < 0.05 \)), but greater than that of Wistar (643 ± 18 mg, \( n = 28 \), \( P < 0.05 \)), despite the fact that the normotensive strains (Wistar and WKY) have similar values of BP (Table 1). Interestingly, the LVM measured in Wistar and WKY were not correlated to the values of BP, whereas this was the case for SHR (Fig. 1). These data indicate that, whereas the LV hypertrophy of SHR seems to be, at least in part, due to pressure overload, the LV hypertrophy observed in WKY is completely independent of this type of cardiac load.

The average LVM normalized by body weight (LVM/BW) of WKY was intermediate between those of SHR and Wistar rats (Fig. 2A). In contrast, the right ventricular mass normalized by BW was not significantly different among the three groups of rats (Fig. 2B), indicating that no right ventricular hypertrophy was detected in SHR or WKY rats.

The cardiac hypertrophy of the WKY rats was also detected after the mass of the left ventricle was examined in vivo by echocardiography. The echocardiographic records of LVM/BW (Fig. 3) revealed that WKY rats exhibited an ~1.4 times higher LVM/BW than Wistar rats; LV hypertrophy was significantly lower than that of the SHR rats. Consistent with these results, the LV end-diastolic wall thickness of WKY rats (1.71 ± 0.04 mm, \( n = 8 \)) was also intermediate between that of Wistar (1.49 ± 0.04 mm, \( n = 7 \), \( P < 0.05 \)) and that of SHR (1.89 ± 0.05 mm, \( n = 7 \), \( P < 0.05 \)). On the other hand, the LV end-diastolic diameter was not significantly different among the three strains (Wistar: 4.9 ± 0.2 mm, \( n = 7 \); WKY: 5.4 ± 0.1 mm, \( n = 8 \); SHR: 5.3 ± 0.2 mm, \( n = 7 \)). Thus the WKY rats exhibit a decreased end-diastolic wall thickness compared with SHR rats.

Table 1. BP and BW values of Wistar, WKY, and SHR rats

<table>
<thead>
<tr>
<th></th>
<th>Wistar (( n = 28 ))</th>
<th>WKY (( n = 41 ))</th>
<th>SHR (( n = 47 ))</th>
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<tbody>
<tr>
<td>BW, g</td>
<td>324.9 ± 9.2</td>
<td>305.3 ± 5.1</td>
<td>314.5 ± 4.5</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>118.5 ± 1.0</td>
<td>119.8 ± 1.6</td>
<td>175.8 ± 1.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), no. of animals. BP, blood pressure; BW, body weight; WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rats. \( *P < 0.05 \), statistically different from Wistar; †\( P < 0.05 \), statistically different from WKY.

Although this study was not designed to explore the cardiac performance of the WKY rats, the echocardiographic studies allow us to make some considerations about this subject. Figure 4A shows that despite the increased BP, the LV fractional shortening (FS) of SHR was not different from that of WKY likely due to the greater wall thickness of the hypertensive strain in the presence of a similar end-diastolic diameter. To rule out possible errors in assessing the FS by measuring the extent of fiber shortening at the endocardial level (12, 32), the mean midwall shortening (12, 32) was also calculated. Similarly, there was no difference between SHR and WKY, albeit the values in both strains were larger than in Wistar (24 ± 1.5, 34 ± 1, and 35 ± 2% in Wistar (\( n = 7 \)), WKY (\( n = 8 \), \( P < 0.05 \)) and SHR (\( n = 7 \), \( P < 0.05 \)), respectively).

Because the values of FS are influenced by cardiac load (22), the differences in afterload must be considered to facilitate the interpretation of the systolic performance in these animals. In this regard, when the FS was normalized by LV end-systolic stress (FS/ESS) (22) the myocardial systolic performance appears to be higher in the WKY than in the other two strains (Fig. 4B) as expected by the fall in the radius-to-thickness ratio in the absence of elevation in BP (6, 17). It is not apparent whether this increased basal cardiac performance of the WKY...
rats is the cause of the hypertrophy. If the FS corrected by ESS is an index of contractility, both SHR and WKY rats exhibit an increased inotropism level compared with the Wistar rats.

Although elevated systemic BP is a very important element in pressure overload-dependent hypertrophy, it is not the only one: any factor that causes LV outflow obstruction can compromise the normal emptying of the LV and therefore lead to hypertrophy. Outflow track impairment can be the consequence of anatomic defects like the disproportionate thickening of the ventricular septum compared with the free wall of the left ventricle, as observed in the asymmetrical septal hypertrophy of humans (with a ratio >1.3) (35). Hearts of the three strains were longitudinally cut along the major LV axis to evaluate the possibility of ventricular outflow obstruction. No disproportionate thickening of the septum was detected because the values of the ratio of septal thickness to LV free wall thickness (measured at 20% of the distance from the aortic valve to the apex) were 0.65 ± 0.1, 0.75 ± 0.05, and 0.73 ± 0.06% in Wistar, WKY, and SHR (n = 4 each), respectively. Alterations in the outflow track can also result from subvalvular, valvular, or supravalvular aortic stenosis. None of these alterations were observed in the WKY rats that we examined as exemplified in the representative histological preparation shown in Fig. 5. In summary, these observations indicate that the myocardial hypertrophy of the WKY rats is not due to pressure overload secondary to obstruction of the outflow track.

Cell capacitance, representative of total cell surface area (TCSA), was ~35% greater in WKY compared with Wistar rats (Fig. 6). Cell capacitance determined in SHR rats was ~20% greater than that of WKY rats, although this difference did not attain the level of statistical significance. In contrast, cell capacitance of SHR was clearly significantly higher (60%) than that of Wistar rats (Fig. 6).

Average cardiomyocyte dimensions are shown in Table 2. Cell width was significantly higher in WKY and SHR than in Wistar rats, whereas cell length was higher in SHR than in the other two strains. In a previous work, Okabe et al. (31) have reported the cardiomyocytes of SHR to be longer and wider than in WKY rats of ages and LVM/BW comparable to those of the rats used in the present study. In contrast, no differences in myocyte width between SHR and WKY rats were found.
The discrepancy in LV tissue specimens was used in their study (31).

Representative micrographs of LV myocardium from Wistar, WKY, and SHR rats are shown in Fig. 7A. An enlarged CSA of the myocytes is observed in the myocardium of the WKY and SHR rats. CSA of myocytes from Wistar was 1.7 and 1.6 times smaller than that of WKY and SHR, respectively (Fig. 7B). The ratio of average major axis (equivalent to cell width) to average minor axis (equivalent to cell thickness) of the myocytes histologically examined was 1.33 for Wistar and SHR and 1.36 for WKY. When we take into account these morphometrically derived values, the cell thickness of the isolated myocytes was estimated from the experimentally measured cell width of these myocytes. Thus, considering the cell as an ellipsoid, we calculated the apparent cell surface area (ACSA) of the isolated myocytes with the following equation

$$ACSA = 2\pi L \left(\left\{(W/2)^2 + (T/2)^2\right\}/2 + 2\pi(W/2)(T/2)\right)$$

where $L$, $W$, and $T$ are the cell length, width, and thickness, respectively. The ACSA was significantly higher in WKY (15%) and SHR (30%) than in Wistar rats (Table 2). The TCSA-to-ACSA ratio (TCSA/ACSA) gives a good idea about the amount of membrane foldings, including invaginations, evaginations, and T-tubules. The values of TCSA/ACSA were 1.28 ± 0.07 ($n = 10$), 1.40 ± 0.08 ($n = 17$), and 1.59 ± 0.12 ($n = 12$) for Wistar, WKY, and SHR rats, respectively. Although no significant differences among the three rat strains were observed, there exists a tendency for higher TCSA/ACSA values in the hypertrophied myocytes, possibly reflecting T-tubule hypertrophy (25).

![Fig. 5](image5.png)

Fig. 5. Representative micrograph of a section along the major axis of the LV of a WKY rat. S, septum; FW, free wall; V, aortic valves; A, aorta. Bar = 2 mm.

![Fig. 6](image6.png)

Fig. 6. Differences in cell capacitance ($C$). Capacitance was recorded by voltage clamping in 20 cells of 10 Wistar, 19 cells of 9 WKY, and 12 cells of 9 SHR rats. Capacitance was higher in WKY and SHR than in Wistar. *$P < 0.05$, statistically different from Wistar.

![Fig. 7](image7.png)

Fig. 7. Cross-sectional area (CSA) of LV cardiomyocytes. A: representative micrographs of cross sections of LV cardiomyocytes of age-matched Wistar (left), WKY (middle), and SHR (right). Magnification ×400. Bar = 20 μm. B: graph showing mean values of CSA obtained after examining with morphometry LV cardiomyocytes of nine Wistar, six WKY, and nine SHR rats. *$P < 0.05$, statistically different from Wistar.

Table 2. Cell Size Parameters

<table>
<thead>
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<th>Wistar</th>
<th>WKY</th>
<th>SHR</th>
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<tbody>
<tr>
<td>$L$, μm</td>
<td>133.6 ± 2.2</td>
<td>135.8 ± 3.3</td>
<td>146.5 ± 2.4*†</td>
</tr>
<tr>
<td>$W$, μm</td>
<td>31.2 ± 0.9</td>
<td>36.7 ± 1.2*</td>
<td>35.2 ± 1.1*</td>
</tr>
<tr>
<td>ACSA, μm$^2$</td>
<td>12,784 ± 464</td>
<td>15,330 ± 655*</td>
<td>15,854 ± 649*</td>
</tr>
<tr>
<td>No. of cells</td>
<td>45</td>
<td>36</td>
<td>54</td>
</tr>
<tr>
<td>No. of animals</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

Values are means ± SE. $L$, length; $W$, width; ACSA, apparent cell surface area. *$P < 0.05$, statistically different from Wistar; †*$P < 0.05$, statistically different from WKY.
Papillary muscles from hypertrophied myocardium (SHR and WKY) were significantly stiffer than those from Wistar, as denoted by the leftward shift of the length-tension curves shown in Fig. 8. Although without reaching statistical significance, papillary muscles of WKY seemed to be more distensible than those of SHR. Nevertheless, these results suggest that normotensive WKY and hypertensive SHR might present, in addition to hypertrophied myocytes, higher levels of myocardial fibrosis than normotensive Wistar rats. To test this hypothesis, the LV CVF was measured in the three strains. Accordingly, interstitial fibrosis is increased in WKY and SHR compared with Wistar rats as evidenced by the representative images of LV myocardium (Fig. 8B) and in the average CVF values (Fig. 8C). In agreement with previous studies (3, 13), mean CVF was significantly higher in SHR than in WKY rats. The difference in CVF between them, however, is too small to explain the difference in LVM.

DISCUSSION

The main finding of this work is that the WKY rat strain presents LV hypertrophy without hypertension. Moreover, the cardiomyocyte hypertrophy and the LV diastolic stiffness of these rats were closer to those of the hypertensive SHR than to those of the normotensive Wistar rats. It can be argued that the myocardial hypertrophy is a feature of the WKY rats inbred in our own animal facility, whereas this is not the case for WKY rats used in other studies. However, the values of LVM/BW of the present study are of a similar magnitude to those widely reported for 12- to 24-wk-old WKY rats (3, 11, 15, 29, 31, 33, 37, 39). Furthermore, the values of LVM/BW of Wistar rats that we are presenting herein are also of similar magnitude as those reported in the literature for Wistar (4, 21, 30) or other normotensive strains (21, 37) different than WKY. These observations strengthen the fact that WKY rats present larger LVM than other age-matched normotensive rats. Our choice of the Wistar rats as the control strain with respect to the WKY rats was based in the fact that the latter was established as an inbred of the Wistar colony from Kyoto that originated the SHR strain (14). However, the possibility of the existence of WKY rats with different degree of hypertrophy from that of the rats used in the present study cannot be ruled out.

The analysis of cell size and morphology revealed that the enlargement of the cardiomyocytes from WKY rats is due to a larger CSA in the absence of a difference in length. These results are consistent with concentric LV hypertrophy (11, 18, 19). On the other hand, the cardiomyocytes from SHR show increased CSA consistent with concentric hypertrophy, but also a slight increase in cell length (7%). It is not apparent whether this enhancement in length is a reflection of the increased LV end-diastolic pressure due to hypertension. This

Fig. 8. A: stress-strain relationship in LV papillary muscles. The muscles were isolated from six Wistar, six WKY, and seven SHR. Papillary muscles from WKY and SHR showed increased stiffness compared with Wistar rats. B: representative microphotographs of LV myocardium of Wistar (left), WKY (middle), and SHR (right). Top, collagen sirius red staining. Middle, collagen selection pattern using the color cube base operation of the color segmentation function using an image analyzer. Bottom, collagen fraction after masking the nonselected objects. Magnification ×400. Bar = 20 μm. C: LV collagen volume fraction (LVCVF) obtained from Wistar (n = 8), WKY (n = 6), and SHR (n = 9). *P < 0.05, statistically different from Wistar; #P < 0.05, statistically different from WKY.
slight cardiomyocyte lengthening was not, however, reflected by the echocardiographic measurement of the diastolic diameter, perhaps as a result of the increased myocardial stiffness that accompanies the hypertensive cardiac hypertrophy.

The hypertrophied myocardium of WKY rats was accompanied by increased myocardial fibrosis, indicated by greater CVF and stiffer myocardium than that of Wistar rats. Other investigators (3, 8, 9) reported stiffer myocardium in SHR than in WKY rats but without comparison with Wistar rats. We would like to emphasize that the stiffer myocardium of the WKY reported herein showed a tendency (although it did not reach statistical significance) to be more compliant than that of the SHR. In any case, we can conclude that the myocardium of the WKY rats is stiffer than the nonhypertrophied myocardium.

Although CSA is similar in SHR and WKY rats, the size of the myocytes is greater in SHR due to the increase in length. This enlargement was also suggested by the measurements of cell capacitance, which was 20% greater in the hypertensive strain, although the difference did not reach a statistical significance. Fibrosis is also greater in SHR than in WKY rats. However, the difference in the levels of CVF between these two strains can hardly explain the difference in LVM.

The pressure overload-independent causes leading to the cardiac hypertrophy and fibrosis observed in WKY rats were not elucidated in the present study. An enhanced expression of the local renin-angiotensin system at LV tissue level might be the explanation for this increased cardiac mass. In connection with this, an increased activity of the angiotensin-converting enzyme in the LV but not in the right ventricle was reported in rats with cardiac hypertrophy (36). Accordingly, previous experiments from our laboratory showed regression of LV hypertrophy in WKY rats after chronic treatment with the angiotensin II type 1 receptor antagonist losartan (2). Moreover, Magga and co-authors (28) also showed that the ratio of heart to body weight was reduced in WKY rats chronically treated with losartan or the angiotensin-converting enzyme inhibitor enalapril. Obviously, the conclusion of whether a pharmacological intervention induces or not regression of myocyte size and/or fibrosis will depend on which control hearts are selected. An inappropriate selection of the “controls” would probably induce to a misinterpretation of the results because a given intervention may decrease these parameters below those of the considered “normal” control hearts.

Slama et al. (39) have recently shown that 28% of the WKY rats that they studied presented biventricular hypertrophy due to ventricular septal defects. However, we did not detect right ventricular hypertrophy in our WKY rats, making unlikely the possibility that the LV hypertrophy observed in our study would be due to ventricular septal defects.

The evaluation of the expression of hypertrophic marker genes across the three strains was not assessed in the present study. However, after searching the literature about data in rats of similar age (17 wk old) and cardiac mass of those used herein, we found that values of plasma levels of atrial natriuretic peptide are increased by twofold in WKY and by more than threefold in SHR (38) compared with Wistar rats (4). These data are in agreement with the observation that WKY rats present intermediate levels of cardiac hypertrophy between those of SHR and Wistar rats.

The plethysmographic tail-cuff method used to record BP in our study represents a limitation. Continuous recording of BP was not employed, ignoring differences in the circadian variations. Studies (41) performed in humans have reported that the blunting of circadian differences in BP (nondippers) was associated with an increase in LVM. Although Descheppe et al. (11) have recently reported that the WKY strain exhibited marked night-morning circadian difference of systolic and diastolic pressure (dippers), studies comparing the circadian variations in the three strains are needed.

In summary, the results of the present study substantiate the use of WKY rats as a pressure overload-independent model of pathological hypertrophy. At the same time, the results raise a concern about the use of this rat strain as an appropriate control for the SHR when pharmaceutical interventions that may affect myocyte size and/or fibrosis are attempted. Future analyses into whether the features of LV hypertrophy observed in WKY rats are linked to defects in specific genes will provide interesting perspectives.

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