Angiotensin Converting Enzyme Variability in Hypertensive and Normotensive Rats

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Recent data have revealed biological and genetic variability in normotensive Wistar-Kyoto rats, which are considered to be the most appropriate control strain for spontaneously hypertensive rats. To investigate the possibility that angiotensin converting enzyme activity could be affected by this variability, we measured plasma and tissue (lung, heart, renal cortex, renal medulla, and adrenal gland) angiotensin converting enzyme activity in spontaneously hypertensive rats and normotensive Wistar-Kyoto rats from three commercial suppliers in France: Iffa-Credo, Janvier, and Charles River Laboratories. Angiotensin converting enzyme activity was measured in vitro with a fluorometric assay using carbobenzoxy-Phe-His-Leu as substrate. Angiotensin converting enzyme activity in both rat strains varied considerably from one supplier to another, and therefore, comparisons of spontaneously hypertensive rats and Wistar-Kyoto rats from the different suppliers produced conflicting results. For Wistar-Kyoto rats, angiotensin converting enzyme activity in the plasma, heart, kidney, and adrenal glands was highest in rats from Iffa-Credo and lowest in rats from Charles River. For spontaneously hypertensive rats, angiotensin converting enzyme activity in the plasma and tissues was highest in rats from Janvier, whereas no difference could be observed between rats from Iffa-Credo and Charles River. These data confirm the problem of how to interpret and compare studies that use spontaneously hypertensive and Wistar-Kyoto rat strains.

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KEY WORDS • kininase II • rats, inbred WKY • rats, inbred SHR

Spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats are widely compared with respect to their cardiovascular phenotypes as models for the study of hypertension. Although both strains have been assumed to be fully inbred, recent data have revealed that biological and genetic variations exist among WKY rats bred in different laboratories, as well as among SHR.1-4 To investigate the possibility that angiotensin converting enzyme (ACE) activity could be affected by this variability, we obtained SHR and WKY rats from three commercial suppliers in France and measured plasma and tissue (lung, heart, renal cortex, renal medulla, and adrenal gland) ACE activities in these rats under standardized environmental and experimental conditions.

Methods

Animals

Eight-week-old male SHR and WKY rats were purchased simultaneously from three different suppliers in France: Iffa-Credo (IC), l'Arbresle; Charles River Laboratories (CR), St. Aubin les Elbeuf, Cleon; and Janvier (JV), Le Genest St. Isle. The three suppliers maintained their rats in specific pathogen-free conditions. IC received their original breeding stocks of SHR and WKY rats from the National Institutes of Health (NIH), Bethesda, Md., in 1976 at the F2 generation for SHR and at the F1 generation for WKY rats. CR received the SHR strain from NIH in 1973 at the F2 generation and WKY rats in 1974 from NIH at the F1 generation. JV started SHR and WKY breeding in 1978 with SHR from the Delalande Research Center (Rueil Malmaison, France), which in turn received their original breeding stocks from Kyoto University (Japan) in 1974; the WKY rats came from the Centre de Sélection et d'Elevage d'animaux de laboratoires (Orléans, France), which received this strain from Kyoto University in 1971. The rats (six per strain and per supplier) were kept 10 days in our laboratory and maintained under the same environmental conditions, i.e., with a standard sodium diet (UAR AO4, 0.4% sodium; UAR, Epinay sur Orge, France) and tap water ad libitum, and air temperature at 22°C and regular 12-hour diurnal cycles.

Plasma and Tissue Sampling

Plasma and tissue sampling was performed on the same day for all 36 rats. The rats were exsanguinated under ether anesthesia by puncturing the abdominal aorta. The blood (5 mL) was collected in a heparinized syringe and centrifuged at 2,000g for 10 minutes (+4°C) to separate the plasma, which was stored at -20°C. The two adrenal glands and fragments of lung (right apex),
heart (apex), and kidney (cortex and medulla) were removed, rinsed in 0.9% NaCl, and quickly frozen in liquid nitrogen before storage at -20°C. After defreezing, the tissues were homogenized in Triton X-100 (0.3%, 100 mg tissue per 1 mL Triton) and centrifuged (11,500g for 20 minutes) after sonification. The supernatants were diluted in Triton X-100 for fluorometric determination of ACE activity.

**Determination of Angiotensin Converting Enzyme Activity**

ACE activity was determined according to the fluorometric method described by Unger et al on the plasma and tissue samples, using an artificial substrate, carbobenzoxy-Phe-His-Leu (Bachem Feinchemikalien AG, Bubendorf, Switzerland). To ensure a linear relation between ACE activity and protein content, the protein concentration in the assay was maintained at less than 2 mg/mL (tissue) or 20 mg/mL (plasma). The protein concentration was determined by the method of Lowry et al using bovine serum albumin fraction V (Sigma Chemical Co., St. Louis, Mo.) as standard. The intra-assay coefficient of variation, determined by assaying 12 aliquots from a pool of plasma or tissue samples, was 3%. In our experiment, the 36 samples from each tissue were handled in duplicate in one experiment.

**Statistics**

Results are expressed as mean±SEM and compared with a two-factor analysis of variance to test for an interaction between suppliers and strains (BMDP statistical programs, BMDP Statistical Software Ltd., Cork, Ireland). This analysis revealed an interaction between the two-factor strains and suppliers that does not allow a multiple comparison of all groups; thus, the differences between rats from the different suppliers for each strain were established by analysis of variance followed by multiple pairwise comparisons. Differences between SHR and WKY rats from each supplier were defined by Student’s t test. A value of p<0.05 was considered significant.

**Results**

Figures 1, 2, and 3 show ACE activity in the plasma, lung, heart, adrenal gland, and kidney (cortex and medulla) in WKY rats and SHR from the different suppliers. Other than in the lung, ACE activity in both rat strains varied considerably from one supplier to another; as a consequence, comparisons of SHR and WKY rats from the different suppliers produced conflicting results. Within strains from a single breeding facility, the coefficients of variation calculated for all tissues were 0.20, 0.23, and 0.09 for WKY rats and 0.19, 0.24, and 0.13 for SHR from IC, JV, and CR, respectively.

For WKY rats, ACE activity in the plasma, heart, kidney, and adrenal gland was highest in IC rats. ACE activity was between two and seven times higher in IC rats than in CR rats. ACE activity appeared to be lower in CR rats than in JV rats, but the differences were not statistically significant.

For SHR, ACE activity in the plasma, heart, kidney, and adrenal gland was highest in JV rats (1.5 to five times higher than CR rats), whereas no difference could be demonstrated between IC and CR rats, with the exception of the renal medulla, where ACE activity in IC rats was higher than in CR rats.

Comparison of SHR and WKY rats within each supplier revealed conflicting results. WKY rats from IC had higher ACE activity in the plasma and tissues (except the lung) than SHR. In rats from JV, ACE activity seemed lower in WKY rats than in SHR, but these differences were not statistically significant in the heart and kidney because of the high variability of ACE activity. In the two rat strains from CR, ACE activity in the lung, heart, and kidney was comparable. In the plasma, ACE activity in WKY rats was significantly higher than in SHR, and in the adrenal gland, ACE activity in SHR was higher than in WKY rats.

**Discussion**

Our results showed that ACE activity in the plasma and tissues (with the exception of the lung) varied between different colonies of SHR and WKY rats. Interindividual variation within a strain from a single breeding facility appeared to be small. This contrasts with results obtained in human populations, in which interindvidual variability is large, because plasma ACE activity can differ between subjects by a factor of up to 5.7. Of course, the results obtained here with a small group of rats are not comparable to a large population.
FIGURE 2. Bar graphs show angiotensin converting enzyme (ACE) activity in heart and adrenal gland from Wistar-Kyoto (open bars) and spontaneously hypertensive rats (hatched bars) from three suppliers: Iffa-Credo (IC), Janvier (JV), and Charles River (CR). Data are mean ± SEM from six rats. In open bars: Significant difference between rat strains (Student’s t test), *p<0.05, **p<0.01. Differences between suppliers (analysis of variance): *p<0.05, **p<0.01.

study. However, over the last 4 years, we have had the opportunity to measure ACE activity in 11 independent groups of 8-week-old WKY rats from IC. Plasma and tissue ACE activity was reproducible with small interindividual variations (e.g., plasma ACE activity of 6.88±0.08 nmol His-Leu per minute per milligram of protein; n=61; minimum, 6.04; maximum, 8.13). These results suggest that among rats from a single breeding facility, ACE activity remains stable.

It should also be noted that in WKY rats, plasma ACE activity was highest in rats from IC, followed by JV and CR. This ranking was also observed in the heart, renal cortex, renal medulla, and adrenal gland. In SHR, ACE activity was always highest in rats from JV, whereas no differences could be observed other than in the renal medulla between IC and CR rats. These observations suggest that the level of ACE activity in the plasma and tissue is controlled in a dependent manner.

Genetic, environmental, and hormonal factors could account for the variations observed among WKY rats and SHR from the three suppliers. The experimental conditions for plasma and tissue sampling and ACE assays were standardized to limit the intra-assay coefficients of variation. In this study, we set out to limit environmental influences by standardizing the conditions in which all the rats were housed during the 10 days in our laboratory. The conditions in our laboratory were not, of course, identical to those of the three suppliers, and it cannot be ruled out that the variations observed here could be the consequence of adaptation to the new housing conditions. However, it appears that such conditions have only a limited effect on ACE activity. Thus, in a previous study, we were able to record age-related variations in ACE activity in SHR and WKY rats born and bred in our laboratory from parents purchased from IC. The plasma and tissues of age-matched rats were comparable to those reported here. Little is known about hormonal control of ACE activity: plasma ACE levels are higher in hyperthyroidism in humans, but, in healthy Caucasian men, no association was observed between ACE activity and plasma T3 level. Triiodothyronin treatment in rats increased renal cortex and renal brush border ACE activity, whereas dexamethasone decreased plasma ACE activity. As we did not measure T3 and cortisol levels in the different rat groups used here, we cannot reject the possibility that different hormone levels might be responsible for ACE variability.

Genetic heterogeneity, as well as biological variability, has been described for WKY rats from different breeding facilities. Clearly, the same could apply to SHR, resulting in problematic interpretations of studies comparing these two strains. The contribution of genetic control in ACE activity level in rats is not evaluated. In humans,
genetic polymorphism accounts for half of the variance of plasma ACE levels. Moreover, the genetic origin of ACE activity variability has been demonstrated in a cross-sectional population study and family study. A family resemblance in plasma ACE levels was demonstrated and may be explained by segregation of a major gene. If a similar pattern exists in rats, it is possible that genetic heterogeneity among WKY rats and SHR available in France may account for variations in ACE activity. Such a possibility must be confirmed by DNA fingerprint analysis and analysis of the ACE loci. It should be noted that Levin et al found that SHR and WKY rat strains from CR had only approximately 50% of their DNA fingerprint bounds in common, whereas we find that ACE activities did not differ between the two strains from these suppliers.

In humans, a weak association was found between serum ACE level and blood pressure. This relation raises the question of a possible ACE role in hypertension or as a marker for high blood pressure in humans. A comparison of ACE activity in WKY and SHR in our study produced conflicting results, with SHR>WKY in rats from JV, SHR<WKY in rats from IC, and SHR=WKY in rats from CR. No clear conclusion could be drawn from the data in the literature. In previous work, we observed that the low level of ACE activity in SHR (IC) was present as early as the third week of life, before hypertension appeared, and that the development of high blood pressure did not modify ACE activity. Nevertheless, it has recently been demonstrated that a gene (BP/SP.1, Bpl) located on chromosome 10 is associated with the development of hypertension. This gene is located near the gene for ACE, which has been proposed as a candidate gene for hypertension. These results were obtained in Heidelberg in Germany and Izumo in Japan by linkage studies of crosses between stroke-prone SHR and WKY rat colonies. But, as the stroke-prone SHR strain has a different genealogy than the SHR, the results obtained on stroke-prone SHR cannot be extrapolated to the SHR strain. Genetic linkage studies involving SHR and WKY rats might be able to identify the ACE gene as a candidate gene for hypertension in SHR. It must also be borne in mind that ACE activity is measured in vitro. It was suggested that in vitro ACE activity is probably not a precise reflection of the in vivo capacity of ACE to generate angiotensin II and that the direct measurement of angiotensin I and II levels may be a better criterion. In fact, Bunkenburg et al found that SHR and WKY rat strains from CR had only approximately 50% of their DNA fingerprint bounds in common, whereas we find that ACE activities did not differ between the two strains from these suppliers.

References

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