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^{1}H NMR-based metabonomic analysis of urine from young spontaneously hypertensive rats

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Abstract

Spontaneously hypertensive rats (SHR) and their substrains are a useful model for studying essential hypertension which is a complex, polygenic, and multifactorial disorder. Their genetic and metabolic features are of great interest because they may provide insights into the mechanism of blood pressure regulation. We have compared urinary metabolic profiles of young SHR with those of their age-matched normotensive controls, Wistar Kyoto rats, using ^{1}H NMR-based metabonomics. Principal components analysis was applied to the NMR spectral data after data-reduced and normalized by the total integral or the creatinine integral. Consequently, a clear separation of urine samples between the two strains was observed in the principal components scores plot. The loadings plot from the data normalized by the creatinine integral showed that many metabolites such as citrate, α-ketoglutarate, and hippurate contributed to the separation, and the urinary levels of most metabolites used in this study, including these three, were lower in SHR than in Wistar Kyoto rats. These metabolic changes may be concerned with blood pressure regulation in SHR, although a relation to other strain differences cannot be ruled out. The present study suggests the usefulness of a ^{1}H NMR-based metabonomic approach using SHR in the field of hypertension research.

Keywords: Hypertension; SHR; Metabonomics; Nuclear magnetic resonance; Urine

1. Introduction

Hypertension is a major risk factor for cardiovascular morbidity and mortality. In the majority of cases, the primary cause of hypertension remains unknown because of its complex nature as a polygenic, heterogenous, and multifactorial disorder, and the disorder is classified as essential hypertension [1,2]. Genetically hypertensive rat models provide a simple and useful model for studying this complex disease [3]. Spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP) are the most widely studied animal models of hypertension because some of the pathophysiological processes are similar to those of essential hypertension. The differences in the genetic and metabolic features between these hypertensive rats and their control normotensive rats, i.e., Wistar Kyoto rats (WKY) are of great interest because they are expected to provide insights into blood pressure regulation in human essential hypertension [4].

Recently, a metabonomic approach using ^{1}H NMR and multivariate statistical analysis has been developed [5,6] to study metabolic differences associated with gene function and pathophysiological and toxicological stimuli. This combined technique has proved extremely successful in terms of toxicity profiling and screening [6]. Although the combined technique has also been applied to the diagnosis of some diseases, its application to understanding pathophysiological processes has been limited [6–10]. Hypertensive patients have been successfully discriminated from normotensive subjects in terms of metabolic profiling by a metabonomic approach using ^{1}H NMR spectroscopic analysis of serums [9]. However, information on what metabolites contribute to this discrimination is considerably limited. Thus, we have undertaken a series of metabonomic studies using hypertensive models. Such animal studies are expected

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to facilitate the observation of metabolic features related to hypertension because the interindividual variation of genetic and metabolic factors is smaller in each strain than in humans, and the experiments can be carried out under the same environmental and feeding conditions. In a previous paper [11], we reported on a urinary metabolic change characteristic in SHRSP with sustained hypertension using a ^{1}H NMR-based metabonomic approach. In this study, we have applied ^{1}H NMR-based metabonomic techniques to characterize the urinary metabolite profiles of young SHR in the early stage of hypertension compared with those of WKY.

2. Materials and methods

2.1. Animals and sample collection

Six male SHR/Izm and WKY/Izm aged 7 weeks were obtained from Disease Model Cooperative Research Association (Kyoto, Japan). Animal studies were conducted under approved guidelines and were reviewed by an institutional animal use committee. The animals were housed at 22 °C with a 12-h light (7 a.m.–7 p.m.) and 12-h dark (7 p.m.–7 a.m.) cycle and had free access to standard chow (CE-2, Clea Japan, Tokyo, Japan) and tap water. Rats were individually placed in metabolism cages (Natume, Tokyo, Japan) to collect urine for 24 h at 8 weeks of age. They were allowed free access to water but no food was given during urine collection. The vessels for urine collection were placed in Dewar vessels filled with dry ice which permitted the collection of urine in a frozen state. Urine samples were filtered through DISMIC-25cs (cellulose acetate, pore size 0.80 μm, Toyo Roshi Kaisha, Tokyo, Japan), and stored at −20 °C prior to NMR spectroscopic analysis. Blood pressure was measured using a tail-cuff method (BP-98A, Softron, Tokyo, Japan) immediately before urine collection.

2.2. ^{1}H NMR spectroscopy

Deuterium oxide (100 μl) containing sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate (TSP, 44 μg) was added to a mixture of urine sample (350 μl) and sodium phosphate buffer (pH 7.4; 0.2 M, 350 μl). The mixture was centrifuged (2250 × *g*, 15 min) and then the supernatant (650 μl) placed in a 5-mm o.d. NMR tube. ^{1}H NMR was measured on a Bruker DPX400 spectrometer at a probe temperature of 300 K with the water resonance suppressed using a NOESYPRESAT pulse sequence [12]. Sixty-four free-induction decays were collected into 32,768 data points with a spectral width of 8013 Hz, a 13 μs pulse width (90° pulse angle), an acquisition time of 2.045 s, and a relaxation delay of 3 s to ensure full T_1 relaxation. Spectra were Fourier transformed after application of a line-broadening function of 0.3 Hz and referenced to a chemical shift of TSP at δ 0.0. Assignments of resonances were made on the basis of literature assignments [13,14] and standard additions.

2.3. Data-reduction of NMR data and principal components analysis (PCA)

All NMR spectra were phased and baseline corrected. Data-reduction of NMR data and PCA were performed using AMIX software (version 3.6.8; Bruker BioSpin, Tsukuba, Japan). The NMR spectra (δ 0.5–9.5) were automatically reduced to 225 integrated segments of equal width (0.04 ppm). The region between δ 4.2 and 6.5 was removed prior to PCA to eliminate the effects of variation in the suppression of the water resonance and in the urea signal caused by partial cross-solvent saturation via solvent exchanging protons. Following the removal of the regions, each integral value of a spectrum was divided by the total sum of the integrals or by the integral intensity of creatinine (δ 4.047–4.067) to normalize each spectral data set. This procedure was performed to compensate for variations in urine volumes. The normalized data sets were then subjected to PCA after mean-centering. Scores plot of the principal components (PCs) were constructed to visualize separation of the urine samples between the groups, and the NMR spectral regions and endogenous metabolites which contribute to the separation were identified from the values of the PC loadings which indicate the importance of each variable to the separation.

2.4. Statistical significance testing

Data are expressed as the means ± S.D. Statistical analysis was done using an unpaired *t*-test. A *p*-value of <0.05 was considered statistically significant.

3. Results

The body weights and blood pressures are summarized in Table 1. The body weights of the SHR were significantly lower than those of the WKY, and the blood pressures of the former were much higher than those of the latter.

Typical ^{1}H NMR spectra of whole urine from the SHR and WKY are shown in Figs. 1 and 2. Although the profiles of the

Table 1
Characteristics of the 8-week-old WKY and SHR[a]

Strain	Body weight (g)	Blood pressure (mm Hg)	
		Systolic blood pressure	Diastolic blood pressure
WKY (n = 6)	228 ± 5	125 ± 3	86 ± 6
SHR (n = 6)	220 ± 7*	167 ± 9**	118 ± 4**

*p < 0.05 vs. WKY; **p < 0.01 vs. WKY.
[a] Values represent mean ± S.D.

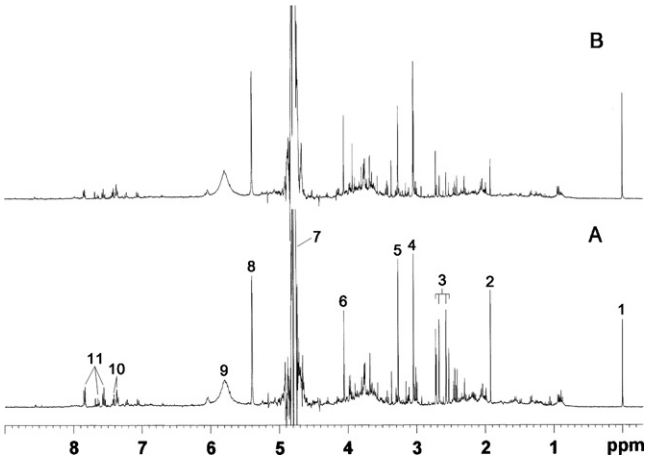

Fig. 1. ^{1}H NMR spectra of urine samples from WKY (A) and SHR (B) at 8 weeks of age. Keys: 1, TSP; 2, acetate; 3, citrate; 4, creatinine; 5, trimethylamine-*N*-oxide; 6, creatinine; 7, H^2HO; 8, allantoin; 9, urea; 10, phenylacetylglycine; 11, hippurate.

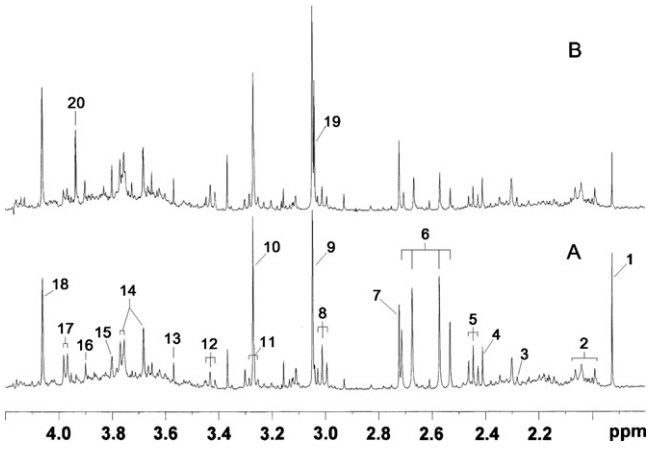

Fig. 2. Partial ^{1}H NMR spectra of urine from WKY (A) and SHR (B) at 8 weeks of age. Keys: 1, acetate; 2, *N*-acetyls of glycoproteins; 3, acetoacetate; 4, succinate; 5, α-ketoglutarate; 6, citrate; 7, dimethylamine; 8, α-ketoglutarate; 9, creatinine; 10, trimethylamine-*N*-oxide, betaine, and taurine; 11 and 12, taurine; 13, glycine; 14, phenylacetylglycine; 15, guanidinoacetic acid; 16, betaine; 17, hippurate; 18, creatinine; 19 and 20, creatine.

NMR spectra had many similar characteristic peaks, differences were observed in the major signals at δ 2.53, 2.57, 2.67, and 2.71 between the NMR spectra from the SHR and those from the WKY on close visual inspection. The signals were assigned to be citrate on the basis of previously published data [6,13]. Further visual comparison of the spectra was difficult because of inter-animal variations in the urine composition, the complexity of the spectra, and differences in the volume of urine excreted. Thus, PCA was applied to the NMR data using two kinds of normalization methods presented in Section 2.

The scores plot obtained from PCA are shown in Fig. 3. The PC1 vs. PC2 plots show that the metabolite profiles of urine samples obtained from the young SHR were clearly different from those from the age-matched WKY, irrespective of the normalization methods. The samples from the hypertensive and normotensive strains were separated along the first PC, with the former having lower scores in the creatinine normalization and higher scores in the total integral normalization.

In order to determine the spectral regions (variables) and metabolites contributing to the separation on the PCA maps, the PC1 loadings, which are a measure of the contribution, were examined. As shown in Fig. 4A, most variables showed plus PC1 loadings in the loadings plot from the data normalized by creatinine, whereas the samples from the SHR had minus PC1 scores in the corresponding scores plot (see Fig. 3A). These results mean that most urinary metabolites in the SHR were excreted in 24 h less than those in the WKY. The spectral regions contributing to the separation between the SHR and WKY are summarized in Table 2, with the assignment of the pronounced signals contained in those regions. By inspection of the PC loadings, together with the ^{1}H NMR spectra of urine samples, the

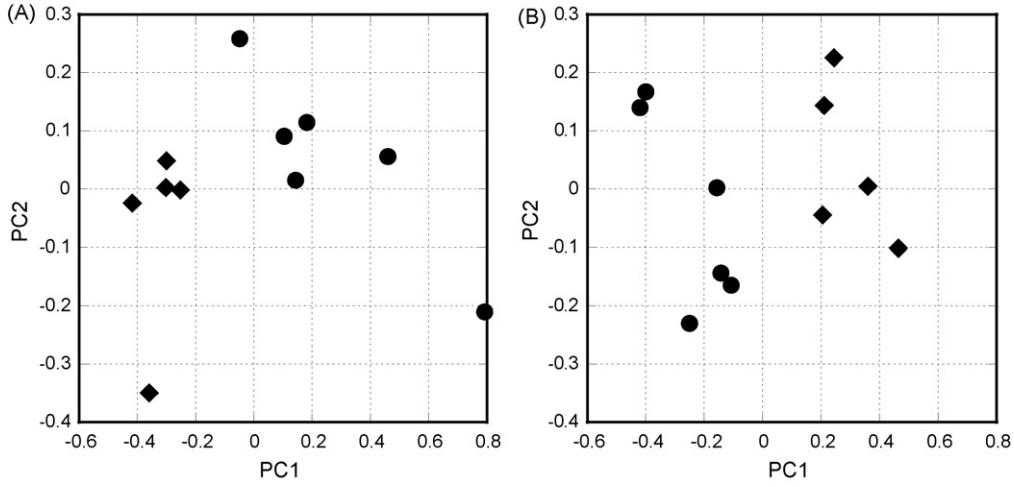

Fig. 3. Score plots generated using PCA of rat urine samples analyzed by ^{1}H NMR. The left plot (A) was obtained from the data after the creatinine normalization and the two PCs captured 76% of the variation (65% for PC1 and 12% for PC2). The right plot (B) was obtained from the data after the total integral normalization and the two PCs captured 71% of the variation (57% for PC1 and 14% for PC2). Filled circle, WKY; filled lozenge, SHR. One spectrum of SHR was eliminated from the calculation because no satisfactory phase correction was obtained for the spectrum probably owing to the slight deviation of the irradiation frequency for water suppression.

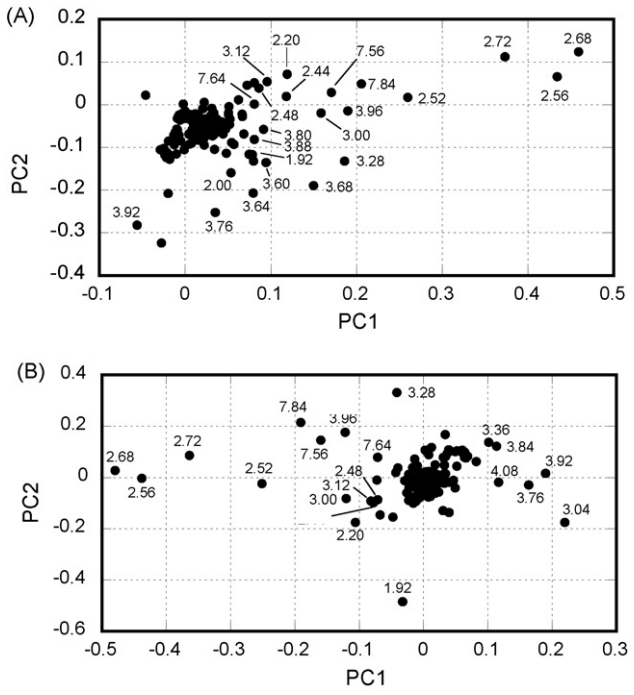

Fig. 4. Loadings plot for the corresponding score plots shown in Fig. 3. The upper (A) and lower (B) plots were obtained using the creatinine normalization and the total integral normalization, respectively. One hundred and sixty-seven variables are shown with the middle δ values of 0.04 ppm regions.

most distinctive metabolic changes seemed to decrease in citrate, α-ketoglutarate, and hippurate, and increase in creatine. The peak intensities of several metabolites listed in Table 2 were measured based on that of creatinine as shown in Table 3. The peak intensities of citrate, α-ketoglutarate, and hippurate were significantly smaller in the SHR than in the WKY. Although the mean peak intensities of acetate, succinate, and taurine in the SHR were relatively small compared with the WKY, the differences were not significant. The variable corresponding to the spectral region δ 3.26–3.30 was also suggested to be lower in the SHR than in the WKY from the loading plots. A prominent and broadened peak appeared at δ 3.27 in the region, which consisted of trimethylamine-*N*-oxide, betaine, and taurine [14]. The intensity of the betaine signal in the SHR was not less than that in the WKY, which was judged from the intensity of another signal due to betaine (δ 3.90). The taurine signal was relatively small, and no significant difference was observed as described above. Thus, the excreted amount of trimethylamine-*N*-oxide was considered to be smaller in the SHR than in the WKY. In addition, an appreciable peak due to creatine appeared at δ 3.04 and 3.93 in four urine samples from the SHR as shown in Fig. 2.

Table 2
Summary of major metabolic differences between SHR and WKY observed in the loadings plot from the data normalized by creatinine intensity

Integrated spectral region[a]	Increase (+) or decrease (−) in SHR[b]	Major metabolite(s) present in selected region (δ, multiplicity)[c]
7.84	−−	Hippurate (7.84, d)
7.64	−	Hippurate (7.65, t)
7.56	−−	Hippurate (7.56, t)
7.52	−	*
7.36	−	PAG (7.35–7.38, m)
7.20	−	*
4.04	−	Cn (4.06, s)
4.00	−	Hippurate (3.97, d)
3.96	−−	Hippurate (3.97, d)
3.92	+	Cr (3.93, s)
3.88	−	Betaine (3.90, s)
3.80	−	GAA (3.80, s)
3.68	−−	PAG (3.67, s)
3.64, 3.60	−	*
3.44	−	Taurine (3.43, t)
3.32	−	*
3.28	−−	TMAO (3.26, s), taurine (3.27, t) Betaine (3.27, s)
3.12	−	*
3.00	−−	KG (3.01, t)
2.72	−−	CA (2.69, d), DMA (2.72, s)
2.68	−−	CA (2.69, d)
2.60	−	CA (2.55, d)
2.56, 2.52	−−	CA (2.55, d)
2.48	−	KG (2.45, t)
2.44	−−	KG (2.45, t)
2.40	−	Succinate (2.41, s)
2.32	−	*
2.28	−	Acetoacetate (2.28, s)
2.24	−	*
2.20	−−	*
2.16	−	*
2.04	−	*N*-Acetyls of glycoproteins
2.00	−	*
1.92	−	Acetate (1.92, s)
1.56	−	*

*Unidentified.
[a] The middle δ value of each region is indicated.
[b] Absolute values of PC1 loadings: +/− = >0.05 and <0.1, −− = >0.1.
[c] Abbreviations: phenylacetylglycine (PAG), creatinine (Cn), creatine (Cr), guanidinoacetic acid (GAA), trimethylamine-*N*-oxide (TMAO), α-ketoglutarate (KG), citrate (CA), dimethylamine (DMA).

No comparison in creatine peak intensities was made because no clear peak was obtained at the chemical shifts in the remainder of the samples owing to small intensity and overlap with other signals.

Table 3
Comparison of urinary excretion of metabolites contributing to the discrimination of SHR from WKY[a]

Strain	Citrate	α-Ketoglutarate	Succinate	Hippurate	Taurine	Acetate
WKY (n = 6)	1.17 ± 0.273	0.40 ± 0.076	0.35 ± 0.056	0.32 ± 0.086	0.19 ± 0.054	0.65 ± 0.456
SHR (n = 5)	0.44 ± 0.100**	0.26 ± 0.081*	0.29 ± 0.059	0.17 ± 0.051**	0.16 ± 0.059	0.38 ± 0.270

*p < 0.05 vs. WKY; **p < 0.01 vs. WKY.
[a] The amounts of citrate, α-ketoglutarate, succinate, hippurate, taurine, and acetate were estimated based on their peak heights at δ 2.57, 3.01, 2.41, 3.98, 3.43, and 1.92, respectively, relative to creatinine peak height (δ 4.06) in individual NMR spectra. Values are mean ± S.D.

Table 4
Summary of major metabolic differences between SHR and WKY observed in the loadings plot from the data normalized by the total integral intensity

Integrated spectral region[a]	Increase (+) or decrease (−) in SHR[b]	Major metabolite(s) present in selected region (δ, multiplicity)[c]
7.84	−−	Hippurate (7.84, d)
7.64	−	Hippurate (7.65, t)
7.56	−−	Hippurate (7.56, t)
6.80, 6.76, 6.72, 6.68, 6.64, 6.60, 6.56, 6.52	+	*
4.12	+	*
4.08	++	Cn (4.06, s)
3.96	−−	Hippurate (3.97, d)
3.92	++	Cr (3.93, s)
3.84	++	*
3.76	++	PAG (3.76, d)
3.72, 3.64	+	*
3.36	++	*
3.12	−	*
3.04	++	Cn (3.05, s), Cr (3.04, s) KG (3.01, t)
3.00	−−	KG (3.01, t)
2.72	−−	CA (2.69, d), DMA (2.72, s)
2.68	−−	CA (2.69, d)
2.60	−	CA (2.55, d)
2.56, 2.52	−−	CA (2.55, d)
2.48, 2.44	−	KG (2.45, t)
2.24	−	*
2.20	−−	*

*Unidentified.
[a] The middle δ value of each region is indicated.
[b] Absolute values of PC1 loadings: +/− = >0.05 and <0.1, ++/−− = >0.1. The PC1 loading corresponding to the region δ 4.02–4.06 including part of creatinine peak was 0.03.
[c] Abbreviations: see Table 2.

In contrast, the loadings plot from the data normalized by the total sum of the integrals yielded fairly different results as shown in Fig. 4B. Many variables were widely distributed to both the left-hand and right-hand sides of the loadings plot. The major spectral regions responsible for the separation between the groups are summarized in Table 4. The separation seemed to be mainly attributed to relatively low urinary levels of citrate, α-ketoglutarate, and hippurate, and relatively high levels of creatine, creatinine, and some unidentified metabolites in the SHR compared with the WKY. Although the major metabolic changes observed in the regions listed in the tables fairly agreed with each other in both normalization methods, inconsistencies were noted in the variables for spectral regions such as δ 1.98–2.06 and 3.62–3.90. The positions of these conflicting variables in the loadings plot, based on the total integral normalization, were shifted to the side of the samples from the hypertensive strain in the corresponding scores plot, compared to their positions in the loadings plot based on the creatinine normalization. Thus, the relation of plus and minus signs of their loadings were mostly reversed between the two normalization methods. In addition, the amount of creatinine excreted in the 24 h urine from the SHR was virtually the same as that from the WKY, as shown in Table 5, when the excreted amounts were measured by a commercial kit (WAKO, Osaka, Japan) based on Jaffe's method as well as the

Table 5
Excretion of creatinine in 24 h urine samples[a]

Strain	Urine volume (mL)	NMR method[b]		Jaffe's method	
		Concentration of creatinine (mg/dL)	Amount of creatinine excreted in 24 h (mg/kg body weight/day)	Concentration of creatinine (mg/dL)	Amount of creatinine excreted in 24 h (mg/kg body weight/day)
WKY (n = 6)	14 ± 5.7	51 ± 11.4	30 ± 6.2	53 ± 12.2	31 ± 3.7
SHR (n = 5 or 6)	15 ± 3.5	47 ± 12.6	29 ± 7.2	47 ± 11.7	31 ± 10.8

[a] Values are mean ± S.D. No significant differences in the values were observed between WKY and SHR.
[b] The excreted amounts of creatinine were measured based on the integral intensities of creatinine signal at δ 4.06 and TSP signal at δ 0.00, the amount of TSP added, and the volume of excreted urine. The values for SHR were obtained from five spectra used for the multivariate analysis.

^{1}H NMR spectroscopy, whereas the loadings plot based on the total integral normalization suggested the relatively high level of creatinine in the SHR. From these observations, it may be conceivable that the integrals of bins, one of which contains creatinine, were overestimated in the SHR when the integrals were normalized by the total sum of the integrals because the total amounts of metabolites in 24 h urine from the SHR was less than those from the WKY. The decrease in the amounts of major metabolites such as citrate and hippurate may be one of the major reasons for the reduction in the total amounts.

4. Discussion

The blood pressures of SHR and WKY rise with growth, and those of the former become significantly higher than those of the latter after 5 or 6 weeks of age, and hypertension is almost established around 10 weeks of age [15]. Thus, 8-week-old SHR are still in the stage of developing hypertension and are useful as a model of the early stage of hypertension. The present experimental results show that the young SHR can be discriminated from the age-matched WKY in terms of their urinary metabolite profiles. The metabolites contributing to the discrimination are of great interest in relation to the mechanisms of blood pressure regulation.

Before NMR spectral data of urine samples are analyzed by PCA, adequate normalization is indispensable in order to remove or minimize the effects of variable dilution of the samples [16,17]. Total integral normalization is generally used for NMR metabonomics, where each integral of the signals or bins of a spectrum is divided by the total sum of the integrals. This normalization assumes that the influence of specific down-regulation of metabolites should be balanced to a certain extent by the up-regulation of other metabolites, and thus the total amounts of metabolites are almost constant, irrespective of gene function and pathophysiological and toxicological stimuli. Normalization is also based on the assumption that renal tubular excretion and reabsorption virtually do not influence the total amounts of urinary metabolites. Additionally, even if the total amounts of urinary metabolites are constant, the elimination of the spectral region, including water and urea signals, may further complicate the situation. Further investigation is thus required concerning the validity of these assumptions in the metabonomic study of young SHR.

It is a common practice to correct concentrations of analytes in urine by the concentration of urinary creatinine in clinical chemistry because creatinine is an indicator of the concentration of urine assuming a constant excretion of creatinine into urine. In addition, creatinine is readily detected as a separate signal at δ 4.06 on the ^{1}H NMR spectra of urine. Thus, creatinine normalization is thought to be practical in ^{1}H NMR-based metabonomic analyses of urine except for special cases such as muscular dystrophy. In the present paper, the urinary excretion of metabolites was compared on the basis of a calculation using creatinine normalization.

As can be seen from the loadings plot (Fig. 4A), many metabolites, notably citrate, α-ketoglutarate, and hippurate, showed lower urinary levels in the SHR compared with those in the WKY, on the basis of creatinine normalization. The metabolites noted in the current work are possibly concerned with blood pressure regulation in SHR although the relation to other strain differences cannot be ruled out. More metabolites possibly related to hypertension could be identified by analyzing urine samples using two-dimensional NMR spectroscopy and ^{13}C NMR spectroscopy as well as by further multivariate statistical analysis of the present ^{1}H NMR spectra. Fujiwara et al. [18] have reported a ^{1}H NMR-based metabonomic analysis for 10-week-old SHR and WKY. They separated the two strains using PCA, and tentatively identified citrate, succinate, and trimethylamine-*N*-oxide as metabolites responsible for the discrimination using soft independent modeling of class analogy. However, no comparison was made concerning the urinary metabolite levels between the two strains. We have investigated younger SHR, which are still in the stage of rising blood pressure, and have identified additional metabolites for the discrimination together with comparison of their urinary levels between the hypertensive and normotensive control rats.

Citrate excretion has been reported to be lower from the SHR at 11–15 weeks of age than from the age-matched WKY due to an increase in citrate reabsorption by renal proximal tubules [19]. It is well recognized that citrate reabsorption increases under metabolic acidosis, in which transported bivalent citrate anion is increased by reduced pH in tubular lumen [20,21]. Indeed, it has been reported that SHR at 6–12 weeks of age are mildly acidotic, which is likely involved in the pathogenesis of the raised blood pressure [22]. Thus, the decrease in citrate excretion, which was observed for the 8-week-old SHR in the present work, can be also explained by the same mechanism as shown here. Additionally, depletion of urinary citrate can be attributed to an impairment of the Krebs cycle [23]. The decreased α-ketoglutarate excretion observed may also be explained on the basis of similar mechanisms shown here because the metabolite is likewise a dicarboxylate intermediate of the Krebs cycle.

Hippurate and trimethylamine-*N*-oxide are readily formed from benzoate and trimethylamine, respectively, in the body, and the latter two compounds originate from the action of intestinal microflora on their precursors such as dietary aromatic compounds and choline [24,25]. The observed differences in the urinary levels of hippurate and trimethylamine-*N*-oxide between the SHR and WKY are thought to reflect the strain differences in the intestinal microfloral populations and their metabolisms that are generally affected not only by diet [25] but also interaction with the host through the exchange and cometabolism of substrates [26]. We further considered that the differences in the intestinal microfloral populations and their metabolism between the hypertensive and normotensive rats might be caused by the differences in the genetic and metabolic factors of the host animals since the two strains were bred under the same circumstances and feeding conditions in the present experiments.

In a previous paper [11], we investigated by an NMR-based metabonomic approach the metabolic differences between SHRSP with sustained hypertension (12 and 26 weeks of age) and age-matched WKY, and the discrimination between the two

strains were found to be mainly due to creatine, taurine, and some unassigned signals, which were different from the present results from the young SHR and WKY. SHR were established by inbreeding WKY with the highest blood pressures [27], and SHRSP were further obtained from SHR, by repeating the selection of the descendants in which one or both parents developed stroke spontaneously [28]. Although the time-course of blood pressure elevation of SHRSP is similar to that of SHR, t he former develop not only stroke but also severe hypertension compared with the latter. Therefore, the comparison of the two kinds of hypertensive models is of great interest in relation to the mechanisms of hypertension and stroke. Further investigation using age-matched SHR and SHRSP is required to explore the differences between the strains because the genetic and metabolic factors probably vary with age.

In conclusion, a metabonomic approach based on ^{1}H NMR spectroscopic urine analysis revealed some characteristic metabolic features of SHR in the early stage of hypertension. Combination with other NMR techniques seems to be useful for the extraction of more metabolic information including identification of the metabolites of interest. Further NMR-based metabonomic studies of various hypertensive rats would provide insights into the fundamental mechanisms involved in the pathogenesis of essential hypertension.

References

[1] N. Kato, Hypertens. Res. 25 (2002) 319–327.
[2] Z. Wu, J. Nakura, M. Abe, J.J. Jin, M. Yamamoto, Y. Chen, Y. Tabara, Y. Yamamoto, M. Igase, X. Bo, K. Kohara, T. Miki, Hypertens. Res. 26 (2003) 533–540.
[3] Y.M. Pinto, M. Paul, D. Ganten, Cardiovasc. Res. 39 (1998) 77– 88.
[4] C.A. Hinojos, E. Boerwinkle, M. Fornage, P.A. Doris, Hypertension 45 (2005) 698–704.
[5] J.K. Nicholson, J. Connelly, J.C. Lindon, E. Holmes, Nat. Rev. Drug Discov. 1 (2002) 153–161.
[6] J.P. Shockcor, E. Holmes, Curr. Top. Med. Chem. 2 (2002) 35–51.
[7] J.L. Griffin, H.J. Williams, E. Sang, K. Clarke, C. Rae, J.K. Nicholson, Anal. Biochem. 293 (2001) 16–21.
[8] J.T. Brindle, H. Antti, E. Holmes, G. Tranter, J.K. Nicholson, H.W. Bethell, S. Clarke, P.M. Schofield, E. Mckilligin, D.E. Mosedale, D.J. Grainger, Nat. Med. 8 (2002) 1439–1444.
[9] J.T. Brindle, J.K. Nicholson, P.M. Schofield, D.J. Grainger, E. Holmes, Analyst 128 (2003) 32–36.
[10] K. Odunsi, R.M. Wollman, C.B. Ambrosone, A. Hutson, S.E. McCann, J. Tammela, J.P. Geisler, G. Miller, T. Sellers, W. Cliby, F. Qian, B. Keitz, M. Intengan, S. Lele, J.L. Alderfer, Int. J. Cancer 113 (2005) 782–788.
[11] K. Akira, M. Imachi, T. Hashimoto, Hypertens. Res. 28 (2005) 425–430.
[12] J.K. Nicholson, P.J.D. Foxall, M. Spraul, R.D. Farrant, J.C. Lindon, Anal. Chem. 67 (1995) 793–811.
[13] M.E. Bollard, E. Holmes, J.C. Lindon, S.C. Mitchell, D. Branstetter, W. Zhang, J.K. Nicholson, Anal. Biochem. 295 (2001) 194–202.
[14] J.C. Lindon, J.K. Nicholson, in: J.C. Lindon, G.E. Tranter, J.L. Holmes (Eds.), Encyclopedia of Spectroscopy and Spectrometry, vol. 1, Academic Press, London, 2000, pp. 98–116.
[15] S. Fukuda, S. Tsuchikura, H. Iida, Exp. Anim. 53 (2004) 67–72.
[16] A. Craig, O. Cloarec, E. Holmes, J.K. Nicholson, J.C. Lindon, Anal. Chem. 78 (2006) 2262–2267.
[17] F. Dieterle, A. Ross, G. Schlotterbeck, H. Senn, Anal. Chem. 78 (2006) 4281–4290.
[18] M. Fujiwara, K. Arifuku, I. Ando, T. Nemoto, Anal. Sci. 21 (2005) 1259–1262.
[19] A. Debska-Slizien, P. Ho, R. Drangova, A.D. Baines, J. Am. Soc. Nephrol. 5 (1994) 1125–1132.
[20] D.P. Simpson, Am. J. Physiol. 244 (1983) F223–F234.
[21] L.L. Hamm, Kidney Int. 38 (1990) 728–735.
[22] P.A. Lucas, B. Lacour, D.A. McCarron, T. Drueke, Clin. Sci. 73 (1987) 211–215.
[23] R.E. Williams, M. Jacobsen, E.A. Lock, Chem. Res. Toxicol. 16 (2003) 1207–1216.
[24] J.L. Smith, J.S. Wishnok, W.M. Deen, Toxicol. Appl. Pharmacol. 125 (1994) 296–308.
[25] A.N. Phipps, J. Stewart, B. Wright, I.D. Wilson, Xenobiotica 28 (1998) 527–537.
[26] J.K. Nicholson, E. Holmes, J.C. Lindon, I.D. Wilson, Nat. Biotechnol. 22 (2004) 1268–1274.
[27] K. Okamoto, K. Aoki, Jpn. Circ. J. 27 (1963) 282–293.
[28] K. Okamoto, Y. Yamori, A. Nagaoka, Circ. Res. 34 & 35 (1974), I-143–I-153.