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LC-NMR identification of a novel taurine-related metabolite observed in ^{1}H NMR-based metabonomics of genetically hypertensive rats

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ABSTRACT

This paper describes the LC-NMR spectroscopic identification of a novel urinary endogenous metabolite responsible for the signals, which were found as major contributors to the separation between genetically hypertensive rats (SHRSP) and normotensive control rats (WKY) in previous NMR-based metabonomic studies. Urine samples from 26-week-old normotensive rats were analyzed by an LC-NMR system equipped with a reversed-phase column having high retention ability for polar compounds. ^{1}H NMR spectra were continuously obtained in the on-flow mode, and the retention times of the unassigned signals in question were determined. Various two-dimensional spectra were subsequently measured for the fraction containing the unassigned signals under the stop-flow mode, which enables for a long accumulation resulting in the enhancement of signal-to-noise ratios. The candidate compound obtained from these LC-NMR data was synthesized, and the NMR and mass spectra were compared with those of the LC-NMR fraction. The unknown metabolite was identified as succinyltaurine from these experiments together with standard addition experiments. This novel metabolite, which is characteristic of the normotensive rats, is very interesting because it is structurally related to hypotensive taurine, and not substantially detected in the genetically hypertensive rats, which excreted more taurine than the normotensive rats. The biological and pathophysiological significances of succinyltaurine remain to be investigated.

1. Introduction

Hypertension is the most common risk factor for cardiovascular morbidity and mortality. The cause of most hypertension remains unknown because it is a complex, multifactorial and polygenic disease, and such disease is classified as essential hypertension [1]. Genetically hypertensive animal models provide a simple means of studying this complex disease [2] because their genetic and environmental factors are relatively homogeneous. Spontaneously hypertensive rats (SHR) and their substrains such as stroke-prone SHR (SHRSP) have been recognized as one of the most useful models for the study of essential hypertension and other related disorders [3]. SHR were established by inbreeding Wistar Kyoto rats (WKY) with the highest blood pressure [4], and SHRSP were further obtained from SHR, by repeating the selection of the descendants in which one or both parents developed stroke spontaneously [5]. As a result of such a selection process, the blood pressures of the SHRSP became higher than those of the SHR. The blood pressures of these hypertensive rats and WKY rise with growth, and those of the hypertensive rats become significantly higher than those of the WKY after several weeks of age without exception, and hypertension is generally established after 10 weeks of age [6]. Thus, the differences in the genetic and metabolic features between these hypertensive models and their normotensive controls, i.e., WKY are of great interest in connection with the pathogenesis and maintenance of essential hypertension. Although many studies have been performed to determine the genes and proteins responsible for hypertension in SHR and SHRSP, the genetic and proteinic bases have yet to be elucidated [7–9].

A metabonomic approach using ^{1}H NMR spectroscopy and chemometric techniques has been generally used to study metabolic differences associated with gene function and pathophysiological and toxicological stimuli [10]. Recently, this approach

Abbreviations: SHR, spontaneously hypertensive rats; SHRSP, stroke-prone spontaneously hypertensive rats; WKY, Wistar Kyoto rats; TSP, sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate; ESI, electrospray ionization; FID, free induction decay; COSY, correlation spectroscopy; HSQC, heteronuclear single-quantum correlation spectroscopy; HMBC, heteronuclear multiple-bond correlation spectroscopy; DOSY, diffusion-ordered spectroscopy.

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has been applied to hypertension in several studies [11–16], and metabolic changes related to the above hypertensive rats were reported. In a previous paper [12], we applied ^{1}H NMR-based metabonomic techniques to the exploration of metabolic differences between SHRSP at 12 and 26 weeks of age and the age-matched WKY. Consequently, the two strains were found to differ on the basis of the urinary metabolic profiles detected by NMR spectroscopy. The metabolites largely contributing to the discrimination were taurine, creatine, and unassigned signals at around δ 2.5, 3.1, 3.4, and 3.6. In SHRSP at both ages, the urinary levels of taurine and creatine were shown to be higher than those in the age-matched WKY, in the loadings plots produced by multivariate statistical analysis. In contrast, the unassigned signals at δ 2.5, 3.1, and 3.6 were comparably detected in all the urine samples from the six WKY at both ages, and they were not substantially detected in any urine samples from the five SHRSP.

In general, signals on ^{1}H NMR spectra of biofluids can be directly assigned on the basis of literature assignments and standard additions, together with basic information such as chemical shifts, coupling patterns, and integral intensities. However, assignments are often difficult when no information for the signals is available in the literature and the signal overlap is extensive, even if two-dimensional NMR techniques are used. In such cases, HPLC directly coupled with NMR spectroscopy, LC-NMR [17–19], is one of the most powerful methods. Currently, this technique is not fully utilized for the identification of endogenous polar metabolites in biological samples because of various limitations concerning sensitivity and chromatographic conditions such as columns and mobile phase. Nevertheless, the technique is expected to contribute to the identification of potential biomarkers and the understanding of the biochemical bases underlying diseases, in combination with NMR-based metabonomics [10].

In this paper, we aimed to identify the above-unknown urinary metabolites discovered in the course of ^{1}H NMR-based metabonomic studies concerned with hypertension. This study presented an example of LC-NMR procedures to identify the endogenous metabolites responsible for the unassigned signals observed on the NMR spectra of biofluids.

2. Materials and methods

2.1. General

Deuterium oxide, HPLC-grade acetonitrile, trifluoroacetic acid, and sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate (TSP) were purchased from Kanto Kagaku (Tokyo, Japan). The chemical structures of the synthesized compounds were confirmed by the electrospray (ESI)-time of flight mass spectrometry on a Micromass LCT spectrometer and an NMR spectroscopy of deuterium oxide solution on a Varian Mercury 300 (Palo Alto, USA). The pH of the sodium phosphate buffer was adjusted using a HORIBA (Kyoto, Japan) F8E pH meter equipped with an electrode 6328-10C. The pH of the urine samples and other solutions was measured using a HORIBA Twin pH B-112 pH meter.

2.2. NMR spectroscopy of whole urine

Urine samples, obtained from male WKY/Izm and SHRSP/Izm at 26 weeks of age in our previous paper [12], were analyzed in this paper. In brief, the 24 h urine samples were collected from the individual animals placed in metabolism cages after measurements of their blood pressures by the tail-cuff method. To urine samples (0.35 ml) was added a 0.2 M sodium phosphate buffer (pH 7.4, 0.35 ml) and the buffered urine samples (pH 7.1–7.3) were mixed with deuterium oxide (0.1 ml) containing TSP as a chemical shift reference (δ_H 0.0). The resulting mixture was centrifuged (2250 × *g*, 15 min), and ^{1}H NMR of the supernatant in a 5-mm o.d. NMR tube was measured on a Bruker DRX500 spectrometer (11.75 T) at a probe temperature of 300 K with the water resonance suppressed using a NOESYPRESAT pulse sequence [20]. The ^{1}H NMR conditions for the urine samples were the same as in our previous paper [12].

^{1}H NMR spectra of whole urine samples were further measured under acidic conditions according to the following procedures. To an aliquot of the WKY urine sample (0.88 ml, pH 6.3) was added 37 μl of an aqueous solution (0.67 M) of trifluoroacetic acid to acidify the sample to pH 3.2. The acidified sample was filtered with DISMIC 13HP (pore size 0.45 μm; Advantec Toyo, Osaka, Japan) to eliminate the precipitated material. The filtrate (0.62 ml) was mixed with deuterium oxide (80 μl), and measured by ^{1}H NMR. Since ^{1}H chemical shift of the ortho position of hippuric acid was considered to be constant in the pH range [21], the left-hand peak of the doublet signal due to the ortho position was used as a reference of chemical shifts and the δ value was set to the same one (δ 7.85) as observed in the above buffered urine samples. The urine samples from the SHRSP were also analyzed in a similar manner as above.

2.3. Synthesis and NMR spectroscopic analyses of 4-oxo-4-[(2-sulfoethyl)amino]-butanoic acid (succinyltaurine)

Triethylamine (3.07 ml) was added to a suspension of taurine (1.25 g) and succinic anhydride (1.00 g) in acetonitrile (10.0 ml) at ambient temperature. Following stirring for 1 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in water and then filtered through Dowex 50W-X8 ion-exchange resin. The filtrate was concentrated under reduced pressure to yield succinyltaurine (quantitative yield) as both colorless and amorphous. The water solution of this free-acid was relatively unstable, and thus it was converted into the mono sodium salt by treatment with sodium dihydrogen phosphate for NMR analysis. ^{1}H NMR (referenced to H^2HO at δ4.70): δ3.42 (t, 2H, –C$\underline{H_2}$–NH–, J = 6.9 Hz), 2.93 (t, 2H, –C$\underline{H_2}$–SO_3Na, J = 6.8 Hz), 2.49–2.54 (m, 2H, –C$\underline{H_2}$–CO_2H), 2.37–2.42 (m, 2H, –NHCO–C$\underline{H_2}$–); ESI-mass: m/z 226.0387 [Calcd for $C_6H_{12}NO_6S$ (free-acid): MH^+ 226.0385], 248.0202 [Calcd for $C_6H_{11}NO_6NaS$ (mono sodium salt): MH^+ 248.0205].

An aqueous solution of succinyltaurine (0.33 mg/ml, 0.45 ml) was mixed with a 50 mM sodium phosphate buffer (pH 7.4, 0.45 ml), and the resulting buffered solution (pH 7.3, 0.7 ml) was analyzed by NMR (NOESYPRESAT) after the addition of deuterium oxide (0.1 ml) containing TSP. In addition, ^{1}H NMR spectra of the buffered solution (0.9 ml) containing succinyltaurine were similarly measured after lyophilization and reconstitution in deuterium oxide (0.9 ml) containing TSP.

2.4. LC-NMR

The LC-NMR system consisted of an Agilent 1100 binary pump, a Hewlett Packard 1100 VWD detector (operated at 210 nm), LCNMR interface BPSU-12, and a Bruker AV500 spectrometer (11.75 T) equipped with a 4-mm flow probe with an effective cell volume of 120 μl. An HPLC column, Atlantis^{TM} dC18 (Waters, MA, USA) (100 mm × 4.6 mm i.d., 5 μm) was used. The outlet of the UV detector was connected to the LC-NMR flow probe via an inert polyetheretherketone capillary (0.25 mm i.d.) so that there was a delay of 1.93 min before a UV peak reached the NMR flow cell. The mobile phase was deuterium oxide containing 0.006% trifluoroacetic acid, with a flow rate of 0.25 ml/min. The mobile phase was changed at 60 min after injection to a mixture of the initial mobile phase and acetonitrile containing 0.006% trifluoroacetic acid (1/1, v/v), and then the column was washed at a flow rate of 1 ml/min

for 8 min. The column was subsequently conditioned by the initial mobile phase at a flow rate of 1 ml/min for 1 min before the next injection. Urine samples (200–300 μl) were injected after being acidified to pH 3.1 or 3.2 and filtered with DISMIC 13HP. NMR was measured at a probe temperature of 302 K. ^{1}H chemical shifts were internally referenced to the residual acetonitrile signal at δ 2.1 because acetonitrile showed a ^{1}H signal at δ 2.056 when the LC-NMR fraction containing the unassigned signals was collected and analyzed in a 5 mm NMR tube after addition of sodium 2,2-dimethyl-2-silapentane-5-sulfonate ($\delta_H = 0$). ^{13}C chemical shifts were internally referenced to the residual acetonitrile signal at $\delta_C = 1.33$.

In the on-flow mode, successive ^{1}H NMR spectra were acquired automatically for 1 h after injection, with each spectrum comprising the summation of 32 free induction decays collected with 8K data points. Other parameters were spectral width, 10,000 Hz; pulse width, 8.00 μs; acquisition time, 0.41 s; relaxation delay, 0.50 s. The total accumulation time of one spectrum was 0.54 min. In order to suppress the signals from the residual water and acetonitrile, the ^{1}H NMR spectra were collected using the WET method [22]. Prior to Fourier transformation, free induction decays were multiplied by a π/2.5 shifted sine-bell-squared function. In the stop-flow mode, the flow of the mobile phase was stopped at 13.8 min after injection, when the portion with the retention time of 11.9 min reached the NMR flow cell, and then the ^{1}H NMR and various two-dimensional NMR spectra, i.e., COSY (correlation spectroscopy), HSQC (heteronuclear single-quantum correlation spectroscopy), HMBC (heteronuclear multiple-bond correlation spectroscopy), and DOSY (diffusion-ordered spectroscopy) [23] were successively measured.

3. Results

In our previous metabonomic study of WKY and SHRSP at 12 and 26 weeks of age, the unassigned signals at approximately δ 2.5, 3.1, and 3.6 (U1, U2, and U4) were observed exclusively in the ^{1}H NMR spectra of the WKY, as shown in Fig. 1 A [12]. Such unassigned signals have not been reported to date in the literature in spite of many ^{1}H NMR-based metabonomic studies. Although COSY experiments of the urine samples from the WKY seemed to indicate a cross peak between the unassigned signals at δ 3.1 and 3.6, their coupling was obscure because of other overlapped signals. Because of the complexity of the spectra, LC-NMR was thought to be necessary to obtain further structural information.

Urine contains various kinds of highly polar metabolites, and they are often acidic or alkaline. When a urine sample from WKY aged 26 weeks was analyzed by ^{1}H NMR after acidification to pH ca. 3, the overall spectral pattern remarkably changed as shown in Fig. 1B. It was thought that most urinary metabolites are relatively stable under such an acidic condition. The unassigned signals at δ 2.5 seemed to shift to δ 2.6 and 2.7 by comparison with the spectrum of the acidified urine sample from SHRSP whereas the chemical shift of other signals (δ 3.1 and 3.6) did not change. Further acidification had no effect on the chemical shift change of the signals. The unassigned signal at δ 3.4 was missing after being acidified. These results imply that the shifted signals, at least, originated from acidic metabolites. Thus, in the following experiments, the urine samples were analyzed by LC-NMR under such an acidic condition to obtain good chromatographic separation at least for the acidic metabolite.

The acidified urine samples were directly analyzed by the LC-NMR system under the on-flow mode without any pretreatments such as extraction. Deuterium oxide containing 0.006% trifluoroacetic acid was used as the mobile phase because the pH value of the 0.006% aqueous solution of the acid was identical to that of the injected urine samples. No appreciable differences were observed in the chromatographic patterns between the WKY and SHRSP in the UV-detected LC-NMR chromatogram.

Unassigned signals (U1, U2 and U4) were sought from the NMR spectra acquired during the on-flow LC-NMR analysis. As shown in the stacked plot (Fig. 2), the NMR spectra at the retention times ranging from 8 to 15 min contained four triplet signals at δ 2.6, 2.7, 3.1, and 3.6, which were presumed to be due to methylene groups based on chemical shifts. These signals were considered to correspond to the above-unassigned signals detected by ^{1}H NMR spectroscopy of the acidified whole urine sample from WKY. These triplet signals were not observed in the on-flow LC-NMR analysis of the urine samples from SHRSP. There seemed to be couplings between the signals at δ 2.6 and 2.7, and the signals at δ 3.1 and 3.6, judging from the signal shapes and the agreement of the coupling constants (~7 Hz) of the four signals. The integral intensities of the four triplet signals were almost the same in each spectrum, and the time-courses of their appearance and disappearance were similar

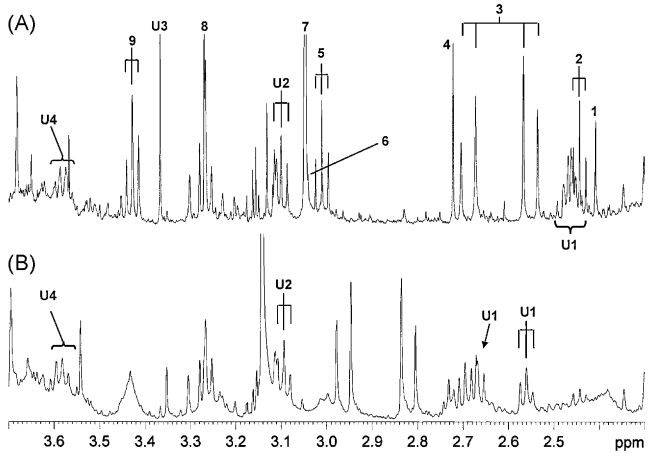

Fig. 1. ^{1}H NMR spectra of buffered (A) and acidified urine sample (B) from WKY at 26 weeks of age. An aliquot of urine (pH 6.3) was buffered to pH 7.3 by mixing with a phosphate buffer (pH 7.4), and another aliquot of the same urine acidified to pH 3.2 by adding trifluoroacetic acid, and their ^{1}H NMR spectra were obtained after the addition of deuterium oxide. Key: 1, succinate; 2, α-ketoglutarate; 3, citrate; 4, dimethylamine; 5, α-ketoglutarate; 6, creatine; 7, creatinine; 8, trimethylamine-*N*-oxide; 9, taurine. Assignments of resonances were made on the basis of literature assignments [33,34] and standard additions. U1, U2, U3, and U4 at approximately δ2.5, 3.1, 3.4, and 3.6, respectively, are unassigned resonances.

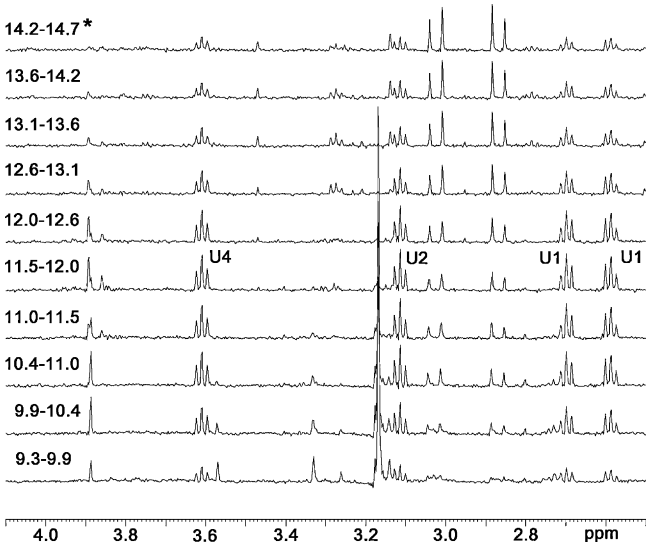

Fig. 2. Stacked plot of an on-flow 500 MHz ^{1}H NMR-detected LC chromatogram of whole WKY urine. *, retention times (min).

to one another.

In order to obtain more structural information, two-dimensional NMR spectroscopy (see the electronic supplementary material) was measured for a relatively long time in the stop-flow mode. For that purpose, the flow of the mobile phase in the above LC-NMR experiments was stopped at 13.8 min after injection when the fraction containing the signals under consideration entered the NMR flow probe. COSY experiments showed that the signals at δ 2.6 and 3.1 correlated with those at δ 2.7 and 3.6, respectively. The results imply that two kinds of moieties with two successive methylene groups exist in almost an equal number of moles. HSQC experiments showed that the ^{1}H signals at δ 2.6, 2.7, 3.1, and 3.6 correlated with ^{13}C signals at δ 30.1 29.0, 49.4, and 34.7, respectively. On the other hand, HMBC experiments showed long-range couplings (two or three bonds) of the ^{1}H signal at δ 2.6 with the ^{13}C signal at δ 29.0, the ^{1}H signal at δ2.7 with the ^{13}C signal at δ 30.1, the ^{1}H signal at δ 3.1 with the ^{13}C signal at δ 34.7, and the ^{1}H signal at δ3.6 with the ^{13}C signal at δ 49.4. Additionally, HMBC experiments showed that both ^{1}H signals at δ 2.6 and 2.7 correlated with both ^{13}C signals at δ 175.4 and 177.3, and the ^{1}H signal at δ 3.6 also with the ^{13}C signal at δ 175.4. All these two-dimensional NMR data suggested a chemical structure of –CH_2–CH_2–X–CO–CH_2–CH_2–CO–. The DOSY spectrum supported, together with the above observations in the stacked ^{1}H NMR plot, that the signals at δ 2.6, 2.7, 3.1, and 3.6 originated from one molecule. Based on the chemical shifts of the ^{1}H signals, the metabolite was thus speculated to be HO_3S–CH_2–CH_2–NH–CO–CH_2–CH_2–COOH.

This compound, named “succinyltaurine” by us, was thus synthesized, and the ^{1}H NMR spectra of the sodium salt were measured in deuterium oxide. Consequently, the spectral pattern was in good agreement with that of the LC/NMR fraction at t_R of about 12 min. Subsequently, the mass spectra of the LC-NMR fraction recovered from the NMR flow cell and the authentic succinyltaurine sodium salt were measured using ESI (positive ion mode), and then peaks at m/z 248.0202 (MH^+) and 248.0208 (MH^+) were obtained, respectively. Both of these spectral data indicated the elemental composition of $C_6H_{11}NO_6NaS$, and thus demonstrated that the LC-NMR fraction contains succinyltaurine sodium salt.

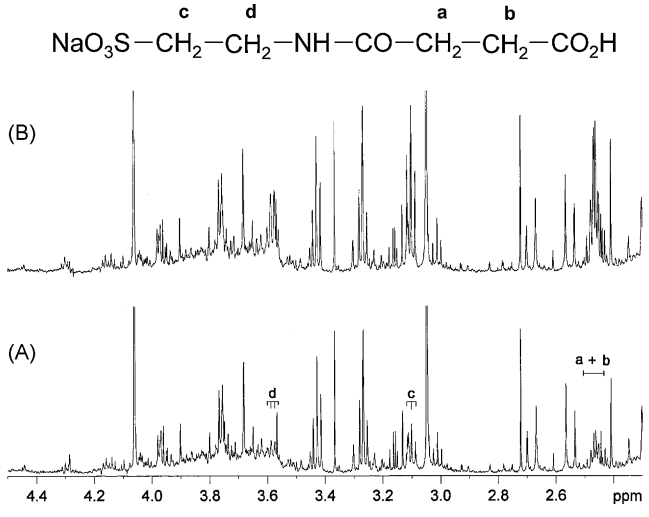

Fig. 3. ^{1}H NMR spectra of the buffered WKY urine sample before (A) and after (B) the addition of authentic succinyltaurine. The authentic succinyltaurine was added to the buffered urine sample analyzed in Fig. 1. The signals a + b, c, and d correspond to U1, U2, and U4 in Fig. 1, respectively. They were assigned to the hydrogen atoms contained in succinyltaurine. The pH-dependent variation of the chemical shifts of the signal U1 is consistent with this assignment. The concentration of succinyltaurine in the excreted urine was estimated to be approximately 0.5 mM based on the amount of authentic compound spiked and the increase in the peak intensities.

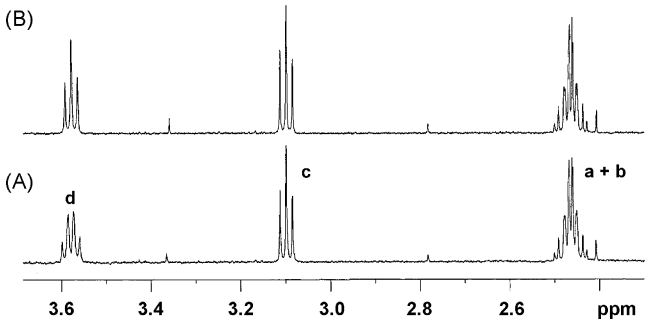

Fig. 4. ^{1}H NMR spectra of authentic succinyltaurine dissolved in a phosphate buffer (pH 7.4) (A), and in a phosphate buffer (pH 7.4) substituted by deuterium oxide (B). The signals a + b, c, and d correspond to those in Fig. 3.

In order to confirm whether the unassigned signals (U1, U2, and U4) shown in Fig. 1A are identical to the signals due to succinyltaurine, a deuterium oxide solution (7.6 mg/ml, approximately 15 μl) of the authentic succinyltaurine sodium salt was spiked to the buffered WKY urine samples used in Fig. 1, and the ^{1}H NMR spectra were measured. Consequently, the peak intensities of the unassigned signals increased by a factor of approximately five without any peak broadening as shown in Fig. 3. From these experiments, the unassigned signals in Fig. 1A were shown to be due to succinyltaurine. The signals, U1, U2, and U3, were assigned to the hydrogen atoms contained in succinyltaurine. Although the signal at δ3.6 appeared as a broad quartet, which differed from the triplet signal in the LC-NMR fraction, the quartet pattern was considered to be due to couplings with the amide proton as well as the adjacent methylene protons in the buffered urine samples. In fact, the signal at δ3.6 also appeared as a quartet when the authentic succinyltaurine was measured by ^{1}H NMR after dissolution in a buffered solution (pH 7.4). However, when measured after the displacement of the buffered solution by deuterium oxide the signal appeared as a triplet, as shown in Fig. 4.

4. Discussion

^{1}H NMR spectroscopic analyses of biofluids are inherently comprehensive, and many signals are readily detected in a short time, for example a few minutes. Thus, a ^{1}H NMR-based metabonomic approach has proved successful in terms of toxicity profiling and disease diagnosis. However, its application to understanding the biochemical processes underlying toxicological and pathophysiological events has been relatively limited probably because many ^{1}H signals are still unassigned and the direct NMR spectroscopic identification of unknown metabolites related to toxicity and diseases in complex biological fluids is difficult. These biochemical studies using NMR-based metabonomics are expected to progress via the effective use of LC-NMR. Although LC-NMR is relatively poor in sensitivity, particularly under the on-flow mode, it has been successfully applied to the identification of drug metabolites in biofluids [17]. In contrast, as far as the authors know, there have been few reports on the analysis of highly polar endogenous metabolites in biofluids by LC-NMR probably because such metabolites are not fully retained on usual reversed-phase LC columns. Recently, some new reversed-phase LC columns have been developed, which can retain highly polar metabolites stronger than conventional ones. Thus, we have applied LC-NMR, equipped with such an improved column, to the structural elucidation of unknown urinary metabolites.

The unassigned signals detected in previous ^{1}H NMR-based metabonomic studies of WKY and SHRSP were found to be due to succinyltaurine based on various spectral data obtained mainly by LC-NMR experiments. The chemical coupling reaction between

taurine and succinate, which are both contained in urine to a considerable extent as shown in Fig. 1, is hard to proceed in the bladder or during sample pretreatments and measurements. In fact, no signal due to succinyltaurine was observed in SHRSP urine which contained succinate and more taurine than in WKY urine. In general, urinary metabolic differences are considered to be due to alteration of the related enzyme activities and renal handling. Because succinyltaurine was exclusively detected in WKY, some enzymes and the corresponding genes responsible for the formation of this metabolite are possibly lacking in SHRSP. When SHR and WKY were bred under the same circumstances in our other studies, succinyltaurine was again detected only in WKY. Therefore, it is likely that the genetic differences concerning succinyltaurine levels were produced when SHR were separated from WKY. Succinyltaurine was observed in only WKY urine, at the earliest, at 8 weeks of age in all of our four independent breeding. Thus, the metabolite may be related to not the initial elevation of blood pressure but the persistence of hypertension.

The biosynthesis and metabolism of taurine were extensively studied using mainly tracer techniques [24]. It is generally accepted that taurine is derived from methionine and cysteine metabolism. Although taurine is well known to be used for bile acid conjugation in the liver of rats and man, it is not incorporated into proteins and generally recognized to be metabolically inert. However, some metabolic conversions have been reported. When [^{35}S]taurine was administered to human subjects, the radioactivity excreted in urine was shown, using an automatic amino acid analyzer coupled with radioactivity detector, to be predominantly due to the unchanged form, and the remainder due to sulfates and 2-hydroxyethane sulfonic acid (isethionic acid) [25]. However, there have been conflicting reports in the literature concerning the biotransformation of taurine in rats [26–28]. When [^{35}S]taurine was administered to rats and the excreted urine was analyzed by TLC, the majority of the radioactivity was due to the unchanged compound and the remainder due to isethionic acid and unidentified metabolite(s), which have been speculated to be sulfates [26]. On the other hand, Sved et al. [27] reported that when ^{14}C-labeled taurine was administered to rats and the excreted urine was analyzed by radio-HPLC, the radioactivity excreted was exclusively due to taurine, showing no metabolites from taurine. In addition, taurocyamine (guanidotaurine) was identified in the urine of rats and man [24,29] although it is ambiguous as to whether the metabolite was formed from taurine. Gamma-L-glutamyltaurine [30] in the brain of mammals and 5-taurinomethyluridine in human mitochondrial tRNA [31] have also been discovered as metabolites of taurine. In spite of these studies, it is possible that succinyltaurine was overlooked because the analytical techniques used in previous studies were insufficient for the comprehensive detection of the related metabolites. Otherwise, this metabolite may be unique to WKY as none of the signals studied here have been reported in the literature although many ^{1}H NMR spectra of urine obtained from various strains of rats have been reported to date. Taurine has also been shown to be excreted in the urine of SHRSP with established hypertension more than in that of the age-matched WKY in our previous metabonomic study [12], whereas succinyltaurine was found here to be substantially detected in only WKY urine. This observation indicates that succinyltaurine may be formed from taurine, leading to the decrease in the level of taurine in WKY. The relationship between taurine and succinyltaurine is of great interest in relation to the pathophysiology of the hypertensive models because these metabolites are structurally related to each other, and taurine is a well-known hypotensive agent [32].

In conclusion, succinyltaurine, a novel taurine-related metabolite in urine, was identified by utilizing LC-NMR coupled with the synthesis of an authentic compound. The biological and pathophysiological significances of this metabolite remain to be investigated. The present report is, to the best of our knowledge, the first to identify novel endogenous metabolites using LC-NMR in NMR-based metabonomics. In addition, this paper provides an example of LC-NMR procedures to identify highly polar metabolites observed on the NMR spectra of biological fluids.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2009.11.006.

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