Further assignment of resonances in ^{1}H NMR spectra of cerebrospinal fluid (CSF)

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Abstract A number of previously unidentified ^{1}H NMR signals detected in CSF spectra of patients with various neurological and metabolic diseases are assigned to metabolites, drugs and drug excipients. Two-dimensional ^{1}H NMR spectroscopy (COSY and J-resolved) is employed to resolve resonances which are hidden by superimposed peaks in one-dimensional spectra. Assignments obtained by making use of 2-D techniques, and of a 1-D ^{1}H NMR data base created for ca. 150 authentic compounds, enable us to clarify the nature of complex signal patterns found in crowded spectral regions of CSF such as the aliphatic methyl region at ca. 1.0 ppm.

Key words: ^{1}H NMR spectroscopy; Biofluid; Cerebrospinal fluid; Signal assignment

1. Introduction

One-dimensional ^{1}H NMR spectroscopy has been used for more than a decade to quantitatively analyze biofluids such as blood serum, plasma, cerebrospinal fluid (CSF), urine, amniotic fluid, etc. [1–12]. Determination of metabolite concentrations in CSF is of particular interest for the diagnosis of neurological diseases [1,6,13]. Moreover, correct NMR signal assignment is a prerequisite for establishing hypotheses on the nature of metabolic perturbations underlying the diseased state. For these reasons, considerable efforts have been made to assign the large number of resonances characterizing high-resolution spectra of CSF and other biofluids. Nevertheless, for many signals detected in CSF samples, assignments have not been available. In a recent publication, we reported significantly changed intensities of unassigned signals for multiple sclerosis vs. control patients [12]. We are now able to present assignments for most of these signals. Additional resonances occasionally detected in our CSF spectra were assigned to drugs or drug excipients.

A great variety of methods and strategies exist for ^{1}H NMR signal assignment, ranging from the simple use of data published in the literature to the application of rather sophisticated pulse sequences for two-dimensional NMR spectroscopy. While some 2-D NMR methods may not be applicable to samples as diluted as CSF, assignments exclusively relying on published chemical shift values may prove to be erroneous due to inconsistent experimental conditions, notably pH. We combined several experimental methods and sources of information including (1) literature values for chemical shifts and coupling constants as a preliminary orientation, (2) use of a 1-D ^{1}H NMR data base consisting of 400 MHz spectra for D_2O solutions of ca. 150 authentic compounds (pH 7.0), most of which were metabolites likely to be found in CSF, (3) spiking CSF samples with authentic compounds, (4) effects of pH variation on 1-D ^{1}H NMR spectra, (5) selective homonuclear decoupling of 1-D ^{1}H NMR spectra, (6) homonuclear 2-D ^{1}H NMR spectra (COSY, JRES) of concentrated CSF samples, and (7) confirmation of NMR assignments by an independent method (HPLC).

Metabolite quantitation is often compromised by significant superposition of NMR signals. For this reason the spectral region between 3.4 and 3.9 ppm (glucose) is rarely exploited in CSF analysis. In contrast, a number of methyl resonances lying in the 'crowded' region between 0.85 and 1.05 ppm have been used for quantitative evaluation based on arbitrary concentration units [12]. We investigated this latter region in more detail by using homonuclear 2-D ^{1}H NMR techniques in order to interpret the spectroscopic pattern obtained by 1-D ^{1}H NMR spectroscopy at 400 MHz for CSF samples at pH 7.0.

2. Materials and methods

2.1. ^{1}H NMR methods

CSF samples were collected by lumbar puncture, lyophilized and prepared for NMR spectroscopy as described previously [12]. ^{1}H NMR spectroscopy for signal assignment was performed by using concentrated CSF samples. Samples from several patients were pooled to reach a concentration factor of ≥10. For one sample, ^{1}H NMR spectra were taken at varying pH values ranging from 2.5 to 7.0, in intervals of ca. 0.5 pH units. Aqueous (D_2O) model solutions containing authentic compounds were prepared and adjusted to pH 7.0. ^{1}H NMR spectra at 400.1 MHz were obtained on a 9.4 T Bruker AM-400 FT-NMR spectrometer using a quattro nucleus probe for 5 mm tubes. Small amounts of sodium 2,2′,3,3′-tetradeutero-3-trimethylsilylpropionate (TSP) were added to the samples to provide chemical shift reference. A standard Bruker BVT-1000 variable temperature unit (VTU) was used to regulate the temperature of the air stream, maintaining a sample temperature of 20°C.

One-dimensional ^{1}H NMR spectra were usually acquired using near full relaxation conditions, i.e. a pulse repetition time (TR) of 13 s, a pulse width (PW) of 14 μs (90°) and an acquisition time (AQ) of 1.36 s (16 k data points), preceded by continuous wave (CW) water proton presaturation for 4.6 s (power setting 14L, ca. 0.035 W). FIDs were multiplied by an exponential function (LB = 0.2 Hz). After zero-filling,

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Abbreviations: AAB, α-aminobutyrate; AHB, α-hydroxybutyrate; AHIV, α-hydroxyisovalerate; AOIV, α-oxoisovalerate; AQ, acquisition time; BHB, β-hydroxybutyrate; BHIV, β-hydroxyisovalerate; Cit, citrate; COSY, correlation spectroscopy; CSF, cerebrospinal fluid; CW, continuous wave; FID, free induction decay; FT, Fourier transform; HPLC, high-performance liquid chromatography; JRES, J-resolved spectroscopy; Lac, lactate; LB, line broadening; NMR, nuclear magnetic resonance; 1,2pd, 1,2-propanediol; PW, pulse width; RD, relaxation delay; S/N, signal-to-noise ratio; SW, spectral width; TD, time domain; TR, repetition time; TSP, sodium 2,2′,3,3′-tetradeutero-3-trimethylsilylpropionate; U, unassigned doublet; UV, ultraviolet; VTU, variable temperature unit; X, unassigned singlet

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Fourier transform, phase and baseline corrections, peak maxima were determined using Bruker's DISNMR peak picking routine based on a polynomial fit procedure.

For selective homonuclear irradiation, Bruker's DISNMR microprogram PRESATHD.AU was employed. Decoupler power was set to 16L = ca. 0.02 W for both water suppression and selective irradiation during acquisition. TR was 7.36 s, including a water suppression period of 6 s before an rf pulse of PW (82°) = 12.8 μs. AQ was 1.36 s (16 k data points).

Two-dimensional ^{1}H NMR experiments were performed by using standard pulse sequences for J-resolved, COSY-90 and COSY-45 spectroscopy. The number of t_1 increments varied between 128 and 512 for COSY experiments. Most COSY spectra were obtained using TD = 512 W in t_1 (1 k in t_2), zero-filled to 1 k (2 k), resulting in a digital resolution of 3.9 Hz/point in both f_1 and f_2 (SW = 4 kHz; 512 t_1 increments). J-resolved 2-D spectra were obtained using TD = 8 k (128 W) for t_2 (t_1), zero-filled to 16 k (256 W), corresponding to a digital resolution of 0.49 Hz/point (0.24 Hz/point). SW was 4 kHz in f_2 (31.3 Hz in f_1 with 128 t_1 increments). Relaxation delay RD was 3 s for all 2-D spectra (including CW water proton presaturation). Two-dimensional spectra were treated with a sinebell window filter function, and were symmetrized after 2-D FT. Spectra were plotted at various intensity levels for analysis, and were evaluated as described elsewhere [14]. Some correlation peaks of weakly concentrated metabolites could not be identified unambiguously due to overlap with large correlation signals from highly concentrated compounds (e.g. glutamine, glucose, citrate), or with t_1 noise originating from these compounds. However, correlation peaks for all coupled methyl resonances assigned in this work were detected without difficulty.

2.2. Spectrum simulation

The spectral region between 0.82 and 1.08 ppm was simulated using EXCEL 4.0 (Microsoft, Redmond, WA) to predict and visualize signal overlap for different magnetic field strengths B_0 between 9.4 and 18.8 T. This limited objective allowed us to choose a simplified simulation algorithm. We assumed Lorentzian line shapes and identical line widths for all signals of this spectral region. Chemical shifts and coupling constants were taken from experimental spectra acquired at 400 MHz for reference solutions and CSF samples. Noise was obtained from a signal free region (0.3–0.7 ppm) of an experimental 400 MHz CSF spectrum, and was added to the calculated spectrum. Digital resolution was kept constant at 0.33 Hz/point for all simulated spectra, a value very close to that of our experimental spectra (0.37 Hz/point). Line widths, peak heights and noise levels were chosen as to generate a simulated 400 MHz spectrum that acceptably matched the experimental spectrum of a typical concentrated CSF sample. Peak heights for the α-aminobutyrate methyl triplet were estimated based on the skyline f_2 projection of a JRES spectrum since this triplet was entirely hidden in the experimental 1-D NMR spectrum. We assumed that all multiplets of the spectral region in question represent pure AX spin systems, resulting in first-order spectral patterns with relative signal intensities (within a multiplet) given by the binomial coefficients of the Pascal triangle. This rule describes the relevant methyl signals in good approximation since the relative chemical shift, $\nu_0\delta$, of each methyl multiplet, i.e. its distance from the multiplet representing the coupled proton(s), is very large compared with the coupling constant, J ($\nu_0\delta/J > 10$) [15]. Consequently, errors for coupling constants and signal intensities do not exceed 5% and 10%, respectively, for magnetic field strengths $B_0 \geq 9.4$ T. The noise level was varied as a function of B_0 to ensure that the signal-to-noise ratio, S/N, was proportional to $B_0^{3/2}$ [15].

2.3. HPLC

For HPLC analysis, 100 μl CSF aliquots were deproteinized at 4°C by ultrafiltration (Ultrafree-MC UFC3LGC-00 Millipore filters for small volumes). Amino acids were derivatized with phenylisothiocyanate, whereas oxoacids were analyzed without derivatization. HPLC analyses were carried out using a Merck Hitachi system (Tokyo, Japan) equipped with an HPLC pump (model L 6200 A) and a variable-wavelength detector (Model LaChrom L7400). For α-aminobutyrate analysis a reversed-phase column was used [16], while α-oxoisovalerate was examined using a cation-exchange Aminex HPX 87A column (Bio-Rad, Richmond, CA, USA) [17]. All assays were run in duplicate.

3. Results and discussion

3.1. Assignment of aliphatic methyl signals

3.1.1. The leucine/isoleucine region. The spectral range defined by the chemical shift values $0.94 < \delta_H < 0.97$ ppm is commonly termed 'Leu/Ile' (leucine/isoleucine) in the biomedical NMR literature [3,18–20]. The resolution usually achieved for biofluid or tissue extract ^{1}H NMR spectra does not permit complete peak separation for this region. We used Gaussian resolution enhancement to analyze these resonances in detail. Since efficient Gaussian apodization is accompanied by a decrease in S/N we used ≥10-fold concentrated CSF to carry out these experiments. This sample concentration resulted in slight δ_H shifts for some signals when compared with samples prepared according to our routine protocol (only 3-fold concentration, owing to the limited availability of CSF from each individual patient). However, these minor chemical shift differences proved to be irrelevant for signal assignments.

Fig. 1 displays the high-field parts of 1-D and 2-D ^{1}H NMR spectra of a typical highly concentrated CSF sample. The methyl region within $0.945 < \delta_H < 0.975$ ppm clearly comprises the two leucine doublets and the low-field peak of the isoleucine triplet, but also the low-field α-hydroxyisovalerate (AHIV) doublet and the high-field peak of the α-aminobutyrate (AAB) triplet. The two lower spectra in Fig. 1 demonstrate that these AHIV and AAB resonances may simply be hidden in the 'Leu/Ile' region of conventional 1-D ^{1}H NMR spectra, in particular when resolution enhancement procedures cannot be applied because of the low S/N typical of routine CSF spectra obtained for samples from individual patients. Our results suggest that the so-called Leu/Ile region should be more adequately named Leu/AHIV/Ile/AAB. However, only 25% of the Ile and AAB methyl triplet intensities lie within this region. Our experience with numerous CSF samples of patients showed that in most cases, only *one* integral value can be obtained for the signal area between 0.94 and 0.97 ppm, representing the *sum* of the signal areas in question. Thus, correct quantitation is considerably complicated for these metabolites. Fig. 1 (bottom spectrum) further demonstrates that the high-field peak of the Ile triplet overlaps with the low-field peak of the α-hydroxybutyrate (AHB) triplet, leaving the central Ile triplet peak as the only signal of this multiplet that can be used for individual quantitation of isoleucine. Moreover, the two low-field peaks of the AAB triplet are completely superimposed with the high-field valine (Val) doublet. This situation renders AAB entirely invisible in 1-D ^{1}H NMR spectra since the CH and CH_2 protons of AAB give signal heights below the detection threshold.

3.1.2. Signal separation by changing experimental conditions. It might be argued that changing sample temperature and/or pH will separate peaks overlapping at 20°C and pH 7.0, and, consequently, will improve signal assignment and quantitation. However, in our experience alterations in these parameters cause increased signal overlap in other spectral regions so that it will be virtually impossible to define temperature and pH conditions optimized for *all* signals. Running several spectra at two or three different temperatures and/or pH values for each patient sample would result in prohibitively long sample preparation, data acquisition and processing times in clinical routine analysis. In addition, repeated CSF manipulation over several hours enhances the risk of sample degradation and contamination, resulting in metabo-

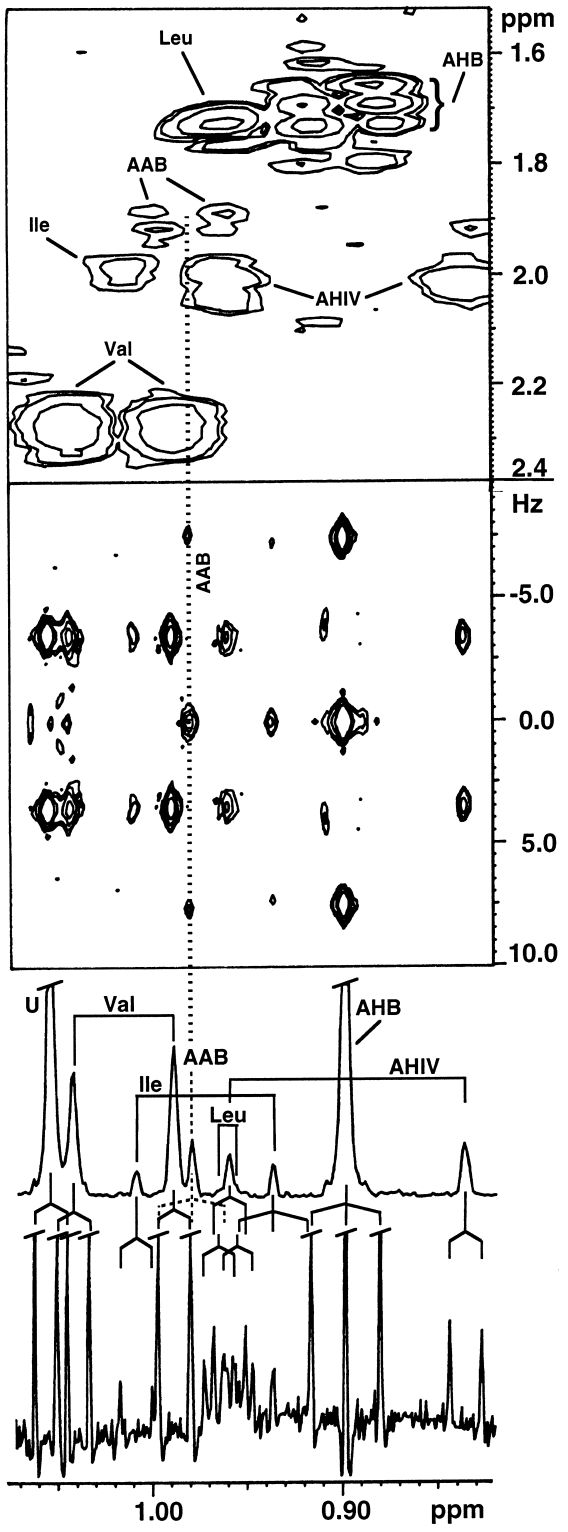

Fig. 1. High-field regions of 1-D and 2-D ^{1}H NMR spectra obtained from a typical concentrated CSF sample. From bottom to top: high-resolution 1-D spectrum processed with Gaussian resolution enhancement, skyline f_2 projection of a JRES spectrum, JRES spectrum, COSY-90 spectrum. The broken line connects the AAB methyl signals. For abbreviations see text.

lite loss and increased quantitation errors. In contrast, the use of ultra-high-field spectrometers will improve resolution for *some* resonances in the critical region between 0.90 and 1.05 ppm, as demonstrated by simulated spectra (Fig. 2). At $B_0 = 14.1$ T (600 MHz, Fig. 2C) the high-field peak of the Ile triplet is isolated from the low-field peak of the AHB triplet, and the Ile doublet does no longer overlap with the high-field Val doublet. At $B_0 = 18.8$ T (800 MHz, Fig. 2D) the Ile triplet is in effect completely separated from the Leu/AHIV region between 0.947 and 0.975 ppm. 600 MHz spectra barely improve the detectability of AAB compared to 400 MHz spectra, while at 800 MHz the central and low-field peaks of the AAB triplet may be detected, but nevertheless are difficult to quantitate due to the intrinsically low AAB concentration in CSF. The two Leu doublets are almost completely separated at 800 MHz while they still overlap with the low-field AHIV doublet. As a result, the use of ultra-high-field spectrometers may significantly improve detection and quantitation of Ile methyl signals, while permitting AAB detection without removing serious peak overlap for the AAB methyl triplet. The low-field AHIV methyl doublet is best isolated from neighboring signals at 600 MHz while it overlaps more severely at 400 and 800 MHz.

3.1.3. Additional methyl signals: confirmation by 2-D NMR. Several methyl resonances lying between 1.1 and 1.5 ppm, and not yet identified in our previous publication [12], were assigned as follows. The doublet at 1.13 ppm, formerly designated XS1.12 and XS1.14 [12], was found to represent α-oxoisovalerate (AOIV). This compound has been previously identified in CSF by a GC-MS method [21], and by 1-D ^{1}H NMR spectroscopy of CSF samples measured at pH 2.5 (1.13 ppm) [11]. AOIV is involved in the synthesis of branched-chain amino acids [22], and was also detected in blood plasma (1.13 ppm, pH 2.5 [10]), urine (1.12 ppm, pH 2.5 [2]) and amniotic fluid (1.12 ppm, pH 9.8 [9]). All of these ^{1}H NMR experiments except one [9] were carried out using lyophilized samples reconstituted in D_2O. Our assignments were confirmed by JRES and COSY spectra (not shown). The correlation signal detected at 1.13/3.05 ppm is consistent with the chemical shift of the CH resonance for the authentic compound (value obtained from our data base). Interestingly, we determined a slightly decreased AOIV chemical shift for pH 2.5 (1.11 ppm, confirmed by a reference solution in D_2O) than the authors cited above, possibly due to the use of ≥10-fold concentrated CSF.

The singlet found at 1.27 ppm (previously named XS1.27 [12]) was ascribed to β-hydroxyisovalerate (BHIV). Direct assignment of this signal is rather difficult due to the absence of correlation signals in COSY spectra. Since BHIV is not commercially available as a model compound, Wevers et al. based their BHIV assignment (singlet at 1.33 ppm, pH 2.5) on GC-MS studies, and on chemical and biochemical considerations [11]. We verified that the singlet observed at 1.27 ppm for pH 7.0 was shifted to 1.33 ppm when the CSF concentrate was gradually acidified to pH 2.5 (Fig. 3). The BHIV methylene singlet (2.55 ppm, pH 2.5 [11]) could not be identified unambiguously in our spectra since in the spectral region at ca. 2.55 ppm, several overlapping signals revealing similar signal areas were detected, each one being on the order of ca. one third of the BHIV methyl peak area (see also Section 3.2).

The alanine methyl region of CSF spectra frequently contains four or five overlapping peaks. For correct alanine (Ala) quantitation it is essential to avoid contamination of the Ala doublet by adjacent signals which may be of the same order of magnitude as the Ala signal itself. The doublet found slightly

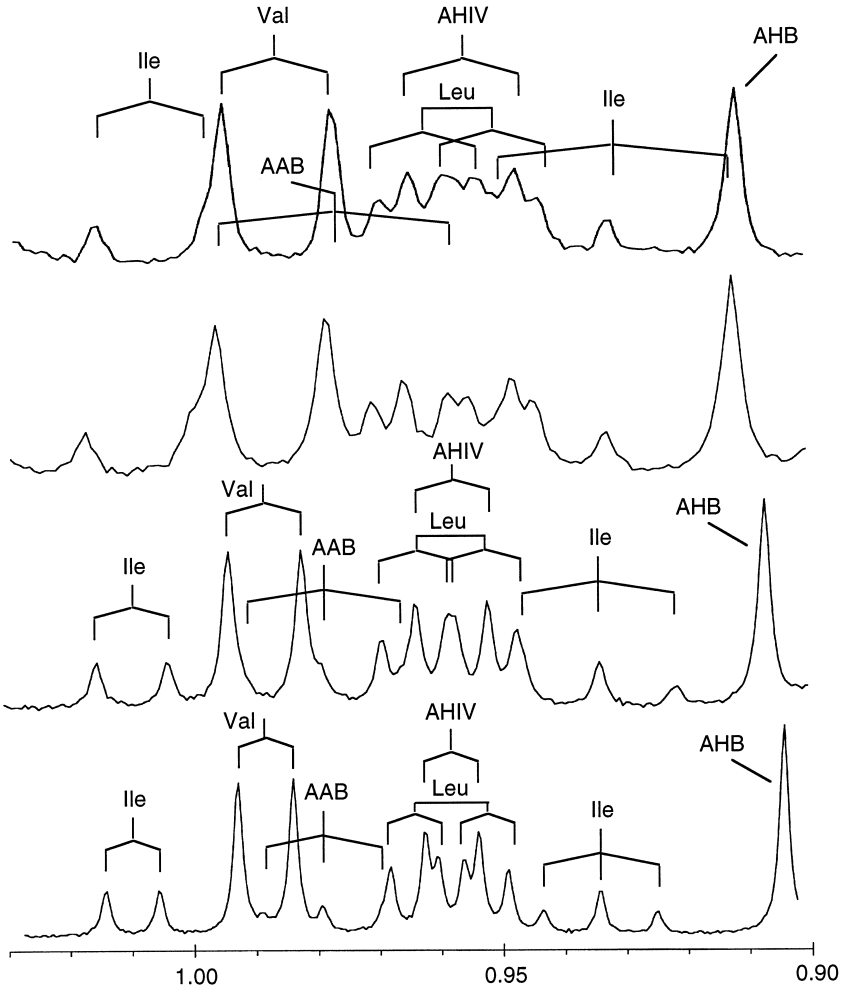

Fig. 2. High-field regions of experimental and simulated high-resolution ^{1}H NMR spectra. A: Experimental 400 MHz spectrum of a concentrated CSF sample. Neither filter functions nor resolution enhancement were applied during data processing. B: Simulated 400 MHz spectrum based on Lorentzian line shapes. Comparison between spectra A and B shows that the experimental spectrum does not exhibit pure Lorentzian line shapes, since most of the signals appear to be broadened at their tops but narrowed near the baseline when compared with the simulated spectrum. C: Simulated 600 MHz spectrum. D: Simulated 800 MHz spectrum.

downfield from alanine is a lactate (Lac) ^{13}C satellite; the corresponding high-field satellite was also detected, with $J_{^1H^{13}C} = 127.8$ Hz and a slight upfield isotope shift of 0.006 ppm. In human CSF, lactate may be as much as 100 times more concentrated than alanine [12]. Thus, despite low natural ^{13}C abundance (ca. 1%) the low-field Lac satellite may result in an overestimation of Ala by $> 50\%$ unless corrected for [11]. The fifth peak 'X' occasionally detected in the Ala region is as yet unassigned.

Resonances formerly marked XS1.14 and XS1.15 [12] were assigned to the 1,2-propanediol (1,2pd) methyl doublet, characterized by a chemical shift of 1.15 ppm and a coupling constant of 6.4 Hz. In COSY spectra, a characteristic correlation peak was detected at 1.15/3.9 ppm. Wevers et al. [11] have identified 1,2pd in ^{1}H NMR spectra of patients' CSF at pH 2.5 (1.13 ppm, consistent with our results; see Fig. 3), but 1,2pd was *not* detected in CSF of untreated healthy or diseased sheep [23]. 1,2pd is a common drug excipient, but may also be involved in glycerol metabolism [22]. Previously, we have demonstrated the presence in CSF of another alcohol, 2,3-butanediol (coupling constant 6.2 Hz), exhibiting the same methyl proton chemical shift as 1,2pd [24]. We were able to separate these two alcohol methyl resonances in COSY spectra while JRES spectra revealed only one methyl doublet due to similar coupling constants [24]. However, the presence in CSF of 2,3-butanediol could be confirmed for *one* patient's CSF sample only, whereas 1,2-propanediol was present in most human CSF samples.

The doublet at 1.07 ppm (previously labeled XS1.06 and XS1.08 [12]) remains unassigned ('U'). A minor contribution to this signal may stem from isobutyrate (a usually unresolved, weak doublet slightly upfield from the main component). Isobutyrate has been detected in human blood plasma by using ultra-high-field ^{1}H NMR spectroscopy [7], but the presence of its methyl correlation peak in our COSY spectrum remains uncertain since we only found intensities very close to the noise level for 1.07/2.4 ppm. In contrast, correlation with a multiplet at 2.5 ppm was regularly observed. The pH dependence of the unassigned doublet 'U' very much resembles that of hydroxy acids such as β-hydroxyisovalerate and lactate (Fig. 3). Therefore, 'U' may represent a hydroxy acid that is not commercially available as an authentic compound. It should be noted that for $pH \leq 5.0$ the methyl resonances of AOIV, 1.2-propanediol and the unknown compound 'U' over-

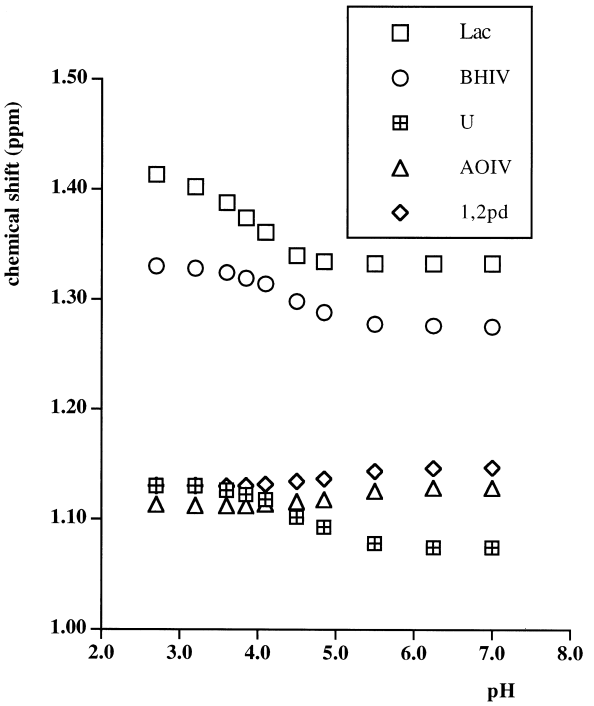

Fig. 3. Chemical shifts of methyl proton signals as a function of pH for several compounds contained in a concentrated CSF sample. The unassigned resonance 'U' shows the same qualitative behavior as the hydroxyacid resonances Lac and BHIV, in contrast to the signals obtained for the alcohol, 1,2pd, and the oxoacid, AOIV.

lap significantly so that the precision of chemical shift determination is compromised.

3.2. Assignment of additional proton signals

Nicotinate and its derivatives are substrates contained in a variety of drugs. Nicotinamide (vitamin PP) plays an important role in the oxidative pathway of cells where it is converted into NAD and NADP. Nicotinamide was detected in the CSF of a patient treated with Nicobion, a drug containing niacinamide (= nicotinamide) as the only active substrate (Fig. 4A, upper spectrum). It exhibits a spectral pattern characteristic of hetero-aromatic compounds; proton couplings were identified by selective homonuclear irradiation. One singlet at 8.93 ppm (a), two doublets at 8.72 and 8.26 ppm (b and c) and one triplet at 7.6 ppm (d) were detected. Closer inspection of the signals revealed the existence of long-range coupling. The singlet was assigned to H2, the doublets to H4 and H6, and the triplet to H5 of nicotinamide. Control spectra for Nicobion, nicotinamide and nicotinate solutions in D_2O were run to confirm the presence of nicotinamide in CSF. Since nicotinamide is prescribed to prevent or treat alcoholic encephalopathies related to vitamin PP deficiency, there is an increased chance of detecting this molecule in CSF of alcoholic patients undergoing vitamin treatment.

Particularly increased 2.50 ppm signal heights were observed for CSF samples from two neurological patients. Closer inspection revealed that in these spectra, the most high-field citrate peak and the low-field part of the glutamine γ-CH_2 multiplet (b) were also increased in comparison with 'normal' spectra, as well as at least three peaks of the glutamine β-CH_2 multiplet (c) (Fig. 4B). Furthermore, a relatively strong additional singlet was found at ca. 4.04 ppm (a). Both patients had been given a drug (Nootropyl) containing piracetam (2-oxo-1-pyrrolidineacetamide) before lumbar puncture. A control spectrum for a piracetam solution in D_2O was acquired to confirm the presence of this compound in the CSF of the two patients. For this control spectrum, a singlet at 4.02 ppm (a), two triplets at 3.52 and 2.50 ppm (b), and a quintet at 2.11 ppm were assigned to piracetam protons (control spectrum not shown). The same signals were identified in the CSF samples in question, with the triplet at 3.52 ppm being obscured by intense glucose signals (Fig. 4B, upper spectrum). The singlet was assigned to the CH_2 protons of the acetamide moiety, the triplets to the 3-CH_2 and 5-CH_2 protons, and the quintet to the 4-CH_2 protons. Piracetam is usually prescribed in myoclonic encephalopathies, or to demented patients. Consequently, these patients should be checked for drug treatment before CSF analysis.

Pyroglutamate (5-oxo-2-pyrrolidinecarboxylate), a product formed by spontaneous intramolecular glutamine cyclization in glutamine solutions not kept deep-frozen, may also reveal a multiplet at 2.51 ppm (pH 7–8 [25]), and may thus add to the signal observed at 2.50 ppm. Further pyroglutamate multiplets have been reported for 4.18, 2.03 and 2.40 ppm [25]. However, we should have only trace amounts of pyroglutamate in our CSF samples since the sample preparation protocol employed ensures that no more than 2–3 h elapse between lumbar puncture and MRS analysis or sample freezing. During most of this time, samples were kept at $\leq$4°C to avoid significant metabolite degradation. Furthermore, as pointed out in Section 3.1, the unassigned methyl doublet 'U' at 1.07 ppm is correlated with a multiplet centered about ca. 2.50 ppm. This multiplet will also contribute to the 2.50 ppm peak. Finally, BHIV which exhibits a CH_2 singlet at 2.55 ppm for pH 2.5 [11] will reveal a slightly shifted CH_2 signal when measured at pH 7.0. Since we were not able to determine the exact position of the BHIV CH_2 peak we cannot exclude that it may add to the 2.50 ppm resonance as well. However, multivariate analysis did not reveal significant correlation between the signal intensities of the BHIV singlet at 1.27 ppm and the peak observed at 2.50 ppm.

3.3. Assignment confirmation by HPLC

AAB could be unambiguously assigned by HPLC. Both retention time (33.2 min) and UV spectrum of the AAB peak were taken as a basis for peak identification; no significant overlap of AAB with adjacent peaks was observed. A characteristic retention time of 3.6 min was found for the peak identified as AOIV, which significantly overlapped with a considerably larger pyruvate signal. Therefore, meaningful UV spectra could not be obtained for AOIV. However, the order of magnitude of the AOIV signal intensity in comparison with that of other identified acids in the same chromatogram was consistent with relative AOIV concentrations as obtained by ^{1}H NMR spectroscopy.

4. Conclusion

Several substituted short-chain fatty acids, one alcohol and two drugs used in neurological treatment were identified in ^{1}H NMR spectra of human cerebrospinal fluid by using one- and two-dimensional NMR methods. This work completes previous CSF studies by making more metabolites available to quantitative analysis, thus enhancing the diagnostic potential

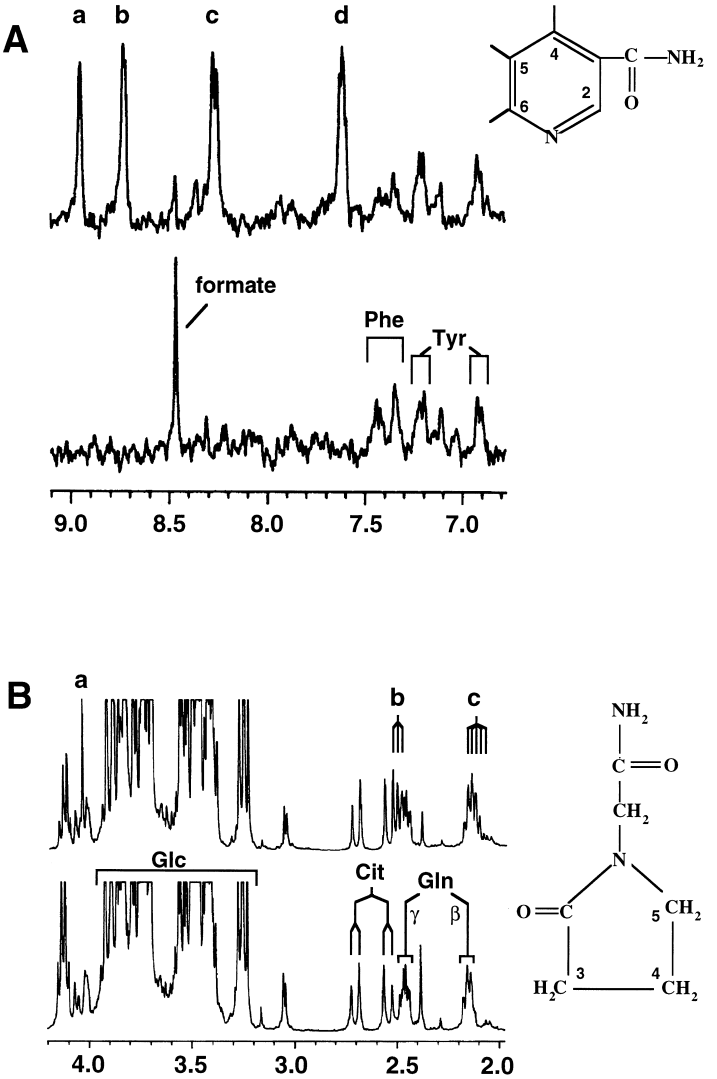

Fig. 4. ^{1}H NMR spectra for CSF samples of patients treated with drugs (upper spectra), compared with spectra from 'normal' CSF samples (lower spectra). A: CSF sample containing niacinamide (= nicotinamide). This spectrum revealed additional lines at 0.9, 1.2 and 2.3 ppm from drug excipients (spectral region not shown). B: CSF sample containing piracetam (= 2-oxo-1-pyrrolidineacetamide).

of CSF tests. Since the concentrations of the compounds assigned in this work have been found to vary significantly between CSF samples taken from different neurological patient groups, these variations may now be used to establish more specific hypotheses on the nature of metabolic changes occurring in neurological diseases, in particular perturbations of amino acid synthesis. The fact that measurable amounts of specific drugs can be detected in some patients' CSF may be exploited for pharmacokinetic studies of molecules crossing the blood brain barrier.

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