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Introduction to clinical in vivo MR spectroscopy

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Abstract

A short introduction to the description of in vivo ^{1}H MR spectra is given. The parameters important for successful measurement and interpretation of spectra are discussed. Basic metabolites visible in proton MR spectra are described and the attention is paid to N acetylaspartate, creatine and cholines.
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Keywords: ^{1}H MR spectroscopy; Chemical shift; Coupling constant; Signal intensity; Metabolism

1. Introduction

Nuclear magnetic resonance (NMR) is concerned with the properties of the spin in the atomic nucleus in condensed matter. Experimental techniques were introduced to physics and chemistry in the middle of the last century [1,2]. It was originally proposed to be used as a method for measuring the basic physical parameters of matter and, subsequently found broad application in chemistry and medicine starting in the early 1950s. At present this method is one of the most important analytical and diagnostic tools in chemistry, biochemistry and radiology.

Due to its non-invasiveness, since the early days of NMR there has been great interest in the application of NMR techniques to the measurement of spectra of living tissue. This first happened in the 1970s when in vivo phosphorus (^{31}P) spectra of tissue were measured in high-resolution NMR instruments [3]. The success of NMR as a medical application started with the introduction of NMR as a diagnostic imaging method [4–6]. Since the 1980s, the abbreviation magnetic resonance (MR) has been used for biomedical applications to reduce any perceived connection with nuclear power. The first in vivo proton (^{1}H) MR spectra of the human brain were published in 1985 [7] and the first clinical applications followed soon. Details on nuclear magnetic resonance are available in many books, as example see [8,9], and electronic teaching materials [10,11]. In this introduction we will focus on a limited number of comments to recapitulate the most important facts needed for the interpretation of spectra.

MR spectroscopy can be considered a method of molecular imaging. Molecular imaging is a diagnostic method based on the observation of cells and molecular structures in vivo using different imaging modalities and new diagnostic and therapeutic markers. The advantage of MR spectroscopy is that it is the only method which allows studying molecular structures in vivo, but, from the point of sensitivity it is one of the worst methods (Table 1). Limited spatial resolution is one of the crucial factors limiting clinical application of MR spectroscopy (Table 2).

Magnetic resonance spectroscopy is focused on in vivo studies in animals and humans. The first step of in vivo MR spectroscopy is MR imaging which enables the choice of the position of the volume of interest (VOI) from which spectra are measured. The result of MR spectroscopy is a set of signals which form a MR spectrum. Their position is described by two axes. This is demonstrated schematically in Fig. 1. The vertical axis represents signal intensity and the horizontal axis serves to describe the signal position in the spectrum on the frequency scale, e.g. (absolute in [Hz] or relative in (ppm)) for qualitative description of signals. With MR spectroscopy we can measure spectra of almost all known isotopes. In vivo biomedical applications are mainly focused on proton (^{1}H), phosphorus (^{31}P) and carbon (^{13}C) isotopes; the following text will focus on ^{1}H MR spectroscopy.

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Table 1
Sensitivity and detection range of molecular imaging techniques (only approximate evaluation)

	Sensitivity	Detection range			
		Organs	Tissue structures	Cells	Molecules
Radionuclide imaging					
Gamma camera	**	+	+		
SPECT	***	+	+		
PET	*****	+	+	+	+
MR methods					
MR imaging	**	+	+	+	
MR spectroscopy	*	+	+	+	+
MR imaging with specific contrast agents	****	+	+	+	
Computer tomography					
CT with contrast agents	**	+	+		
Optical imaging					
Fluorescence	*****	+	+	+	+
Bioluminiscence	*****	+	+	+	+

Table 2
Spatial resolution of MR imaging and spectroscopic methods

	1D resolution [mm]	3D resolution [mm^3]
MR microscopy	0.04–0.005	0.05
Mini MR imaging[a]	0.04	0.1
MR imaging[b]	0.25–1	0.5–1
Mini MR spectroscopy[a]	1–5	20–100
^{1}H MR spectroscopy[b]	5–20	1000–4000
^{31}P MR spectroscopy[b]	20	8000

[a] Small animals.
[b] Whole body systems.

2. Analysis of MR spectra

The measurement of in vivo MR spectra on clinical scanners is a mostly an automated routine which performs the measurement of spectra by single voxel or spectroscopic imaging techniques using pulse MR methods. In this procedure radiofrequency pulses and gradients of the magnetic field enable obtaining the MR spectrum in the time domain. The user has the possibility to control the quality of spectra via changes in the parameters of the pulse sequence. When a spectrum in the time domain is measured, several post-processing methods are available to obtain the spectrum in the frequency domain which is used for interpretation. Procedures of measurement and evaluation of MR spectra are described in detail in other parts of this issue [12–14]. The basic features of MR spectra are described in the following paragraphs.

Signals in MR spectrum (Fig. 1) are described by a few basic parameters:

(a) Chemical shift;
(b) Signal multiplicities;
(c) Signal intensity.

2.1. Chemical shift

In principle, the majority of elements can be studied by magnetic resonance techniques because we can find suitable isotopes with a nuclear magnetic moment which are able to absorb radiofrequency energy (with few exceptions). The basic equation of magnetic resonance is Larmor's equation (resonance

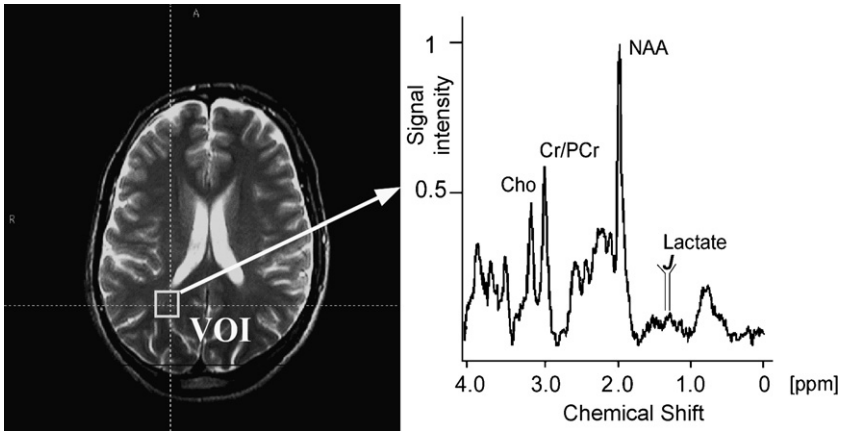

Fig. 1. ^{1}H MR spectrum is measured from the volume of interest (VOI) based on MR images and characterized by chemical shift, signal intensity and coupling constants (J). (The spectrum was measured using STEAM sequence with TE = 10 ms, TR = 5000 ms).

condition) and tells us that the resonance frequency ν_0 (Hz) is proportional to the static magnetic field B_0 (T)

$$\nu_0 = \frac{\gamma}{2\pi B_0} \quad (1)$$

The proportionality constant γ is called gyromagnetic ratio, a natural constant specific for each isotope. Biomedical MR research is focused on studies based on measurement of resonance frequencies of protons, and phosphorus and carbon (^{13}C) atoms which are among the most important nuclei in living tissue. At a magnetic field of 1.5 T the resonating Larmor frequency of a proton is 63.8 MHz and because the gyromagnetic ratio for ^{31}P is much lower than for a proton, the resonance frequency is 25.8 MHz, which means the difference between resonances of hydrogen and phosphorus nuclei is measured in MHz. The application of a higher magnetic field also means that the resonating nucleus absorbs a higher frequency and differences between the resonance frequencies are even greater.

Eq. (1) was proposed for a single (isolated) nucleus not influenced by neighboring nuclei and electrons. In reality such a situation is not valid for chemical compounds because atoms are parts of molecules and connected via chemical bonds. This means that in addition to the external magnetic field each nucleus is influenced by a local magnetic field which is the sum of all neighboring small magnetic fields produced by electrons of chemical bonds. The resulting secondary field has the opposite direction than B_0 and, therefore, a shielding effect. Higher shielding means that the nucleus resonates at a lower resonance frequency. This phenomenon describes screening constant σ characteristic for each nucleus in specific chemical conditions.

The description of various resonance frequencies in the absolute frequency scale (in Hz) is not very practical since it depends on B_0 which is easily varied. So the ppm chemical shift scale (δ scale) concept was adopted. The resonance frequency of nucleus i is calculated in the relative scale as the difference between frequency ν_i and the frequency of reference ν_{ref} divided by the frequency of reference which is in practice replaced by the spectrometer frequency ν_{spc}:

$$\delta_i = \frac{10^6(\nu_i - \nu_{ref})}{\nu_{ref}} \approx \frac{10^6(\nu_i - \nu_{ref})}{\nu_{spc}} \quad (2)$$

where δ_i is a dimensionless parameter which does not depend on the spectrometer frequency ν_{spc}. The position of the signal i to the signal of reference is expressed in parts per million (10^{-6}) and is called chemical shift. There are important conventions applied in this concept. When a signal is found with a higher chemical shift than the chemical shift of the reference, it means that the nuclei resonate at a higher frequency than the nuclei of the reference.

By convention the common reference signal in chemistry is the signal of methyl groups of a synthetic compound, tetramethylsilane (and its derivates) and the position of methyl signals is considered 0 ppm (in principle, signal with the highest known shielding in ^{1}H NMR spectra). In vivo MR spectroscopy usually uses the methyl signal of *N*-acetylaspartate resonating at 2.02 ppm (recalculated from in vitro measurements with the standard tetramethylsilane) as the reference. Table 3 summarizes chemical shifts of the most important metabolites studied using ^{1}H MR spectroscopy.

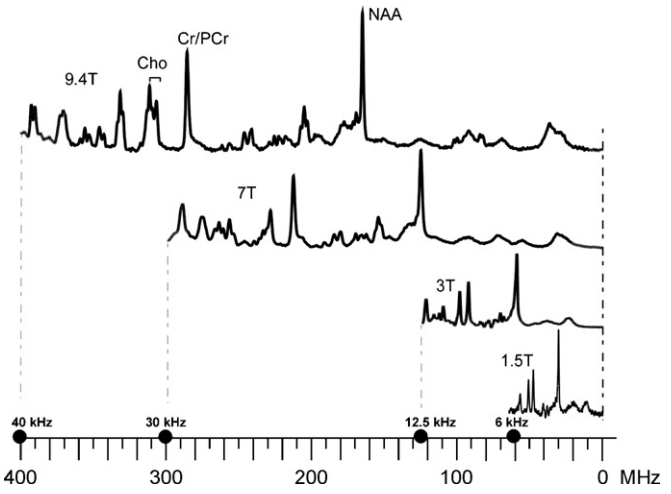

Fig. 2. ^{1}H MR in vivo spectra measured at different magnetic fields. A higher field means a higher difference between signals in Hz. Signal positions in the δ scale [ppm] are the same (Human brain at 7 T and rat brain at 9.4 T, spectra were provided by I. Tkac, University Minnesota). *Comment*: in contrast to the differences between various isotopes which are in the range of MHz, differences due to a change in local magnetic fields are in Hz.

Eq. (2) also means that the highest possible magnetic field is the best for separation of signals in the spectrum. This is demonstrated in Fig. 2. We can see that the separation of signals is much better in a high field than in a low field. On the other hand, we have to keep in mind that even more important than the intensity of the magnetic field is its homogeneity which is characterized by the half-width of the signal. In order to get good spectra in in vivo MR spectroscopy, the signal of water at 1.5 T should have line width of about 10 Hz (Fig. 3).

2.2. Signal multiplicities

Besides the shielding effects (diamagnetic or paramagnetic) which are responsible for chemical shifts, nuclei are influenced by magnetic fields associated with the presence of neighboring spins. Two mechanisms of interaction between the nuclei are possible: (a) a direct through-space interaction depending on the distance between nuclei and (b) an indirect mechanism which is dependent on bonding electrons. These interactions are described by the coupling (interaction) constant J_{ij} in units of Hz. In chemistry both mechanisms are very useful in the interpretation of spectra, especially in the case of liquid samples. Using coupling constants is one of the most important tools for the assignment of signals to appropriate chemical structure.

In in vivo proton MR spectroscopy the visibility of these interactions is rare. This is due to the fact that there are a limited number of metabolites studied by in vivo spectroscopy and these compounds are in a mixture where the observation of interactions, much smaller than chemical shifts, is difficult because all signals are broad.

Nevertheless, the through-space interaction is often observed in methyl signals of creatine in muscles and this interaction

Table 3
Chemical shifts of most frequent metabolites observed by ^{1}H MR spectroscopy

Metabolite	Assignment	ppm (63 MHz)	ppm (400 MHz)*
Lipids (Lip)	CH_3, CH_2	0–2	
Lactate (Lac)	CH_3	1.33 (d)	1.28 (d)
	CH	4.07 (q)	4.07 (q)
Alanine (Ala)	CH_3	1.48 (d)	1.48 (d)
	CH	3.83 (q)	3.70 (q)
N-acetylaspartate (NAA)	CH_3	2.01	2.01
	CH_2	2.62 (t)	2.92, 2.93 (d, d)
	CH	–	4.74 (t)
Glutamate (Glu)	β-CH_2	2.13 (m)	2.13 (m)
	γ-CH_2	2.36 (m)	2.70 (m)
	α-CH	3.79 (t)	3.80 (t)
Glutamine (Gln)	β-CH_2	2.13 (m)	2.11 (m)
	γ-CH_2	2.46 (m)	2.70 (m)
	α-CH	3.81 (t)	3.75 (t)
γ-Aminobutyrate (GABA)	β-CH_2	1.88 (m)	1.84 (m)
	γ-CH_2	2.26 (m)	2.23 (t)
	α-CH_2	2.95 (t)	2.95 (t)
Aspartate (Asp)	β-CH_2		2.98 (m)
	CH		4.06 (q)
Creatine/phosphocreatine (Cr/PCr)	CH_3	3.01	3.01
	CH_2	3.90	3.90
Choline compounds (Cho)	N–$(CH_3)_3$	3.23	3.23
	CH_2	3.76 (t)	3.74 (q)
Taurine (Tau)	N–CH_2	3.37 (m)	3.24 (t)
	S–CH_2	3.37 (m)	3.41 (t)
Glucose (Glc)	$H_{2–6}$	3.45 (m), 3.82 (m)	
Myo-inositol (myo-Ins)	H_5	3.27 (m)	3.25 (t)
	$H_{1,3}$, $H_{4,6}$	3.56 (m)	3.50, 3.52, 3.60 (d, d, t)
	H_2	4.10	4.04 (t)
Scyllo-inositol (scyllo-Ins)	H_5	3.35	
Phenylalanine	Phenyl ring	7.3	7.2–7.4 (m)
Histamine, Histidine	Imidazol ring	7.9	7. 85 (d)
		7.1	7.05 (m)

Resonance assignments with chemical shifts of selected metabolites observable at 63 MHz and 400 MHz in H_2O and D_2O* (internal standard DSS, at 63 MHz there are a maxima of multiplets).

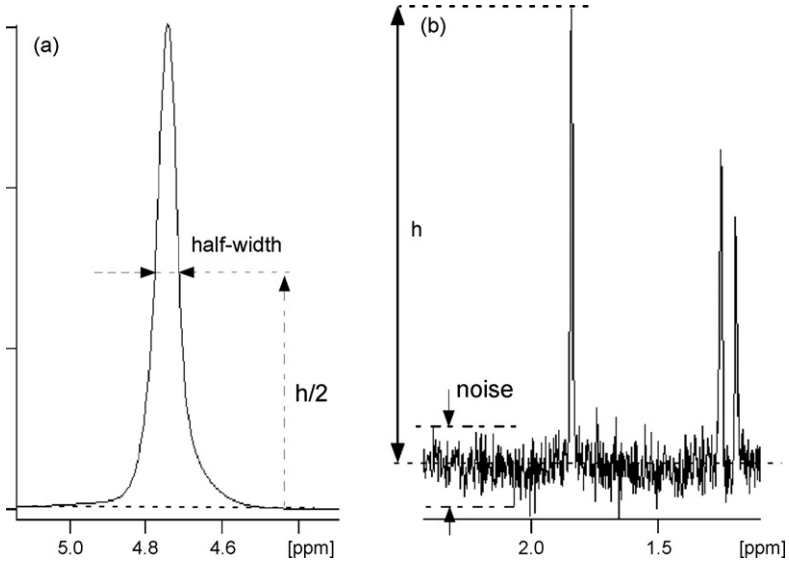

Fig. 3. Half-width (FWHH, full width at the half height) of the water signal (a) and signal-to-noise (S/N) ratio (b) are basic parameters used for the characterization of spectral quality. The half-width of the water signal depends of many factors and good in vivo ^{1}H MR spectra of the brain are obtained when half-widths are about 10 Hz (good shim at 1.5 or 3 T). Signal-to-noise ratio can be easily calculated using the equation S/N = 2.5 h/noise (noise, peak to peak distance in a range of about 100 points without signals, h, height of the signal, 2.5 is the constant).

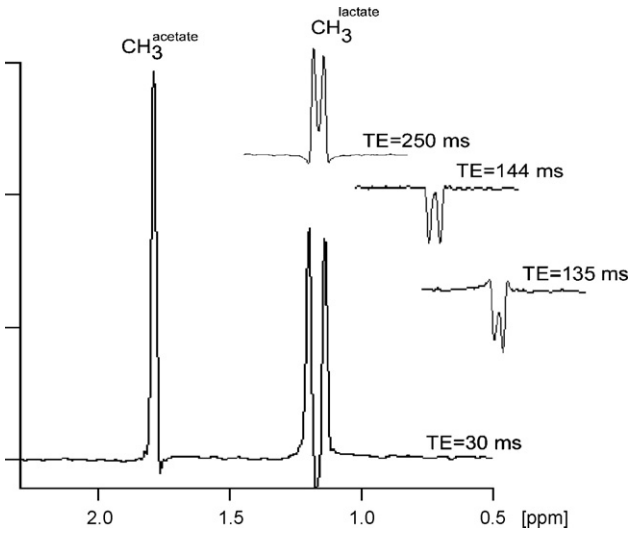

Fig. 4. The spectrum of a lactate and acetate mixture (water solution) which is often used for shim tests of MR imagers and setting of editing MR techniques. The splitting of the CH_3 signal of lactate should be the highest possible. The phase of the CH_3 doublet of lactate strongly depends on echo time TE (spectra were measured at 3 T using the spin echo sequence).

depends on the distance of nuclei in interaction and their orientation to B_0. For details see [15].

The indirect mechanism, responsible for the splitting of signals, is the example of lactate which is very often observable in vivo. This is the case of "weakly coupled system" characterized by high value of the ratio $(\nu_i - \nu_j)/J_{ij}$, where ν_i and ν_j are chemical shifts of nuclei i and j in Hz and J_{ij} is the interaction constant in Hz. The multiplicity x of the signal which results from spin coupling with n equivalent nuclei of spin I is given by the following equation:

$$x = 2nI + 1 \tag{3}$$

In the case of lactate, the interaction between CH_3 equivalent protons and C–H is characterized by a doublet with $J = 6.9$ Hz at 1.33 ppm (Fig. 4). In the solution we can also see the quartet of CH groups of lactate at 4.11 ppm. Contrary to chemical shift measured in Hz, interaction constants J are not dependent on the magnetic field used by the spectrometer.

The case of a strongly coupled system (the ratio $(\nu_i - \nu_j)/J_{ij}$ is close to 1), can be predicted only by quantum mechanical calculation. It should be mentioned that in normal brain tissue the concentration of metabolites where we can observe J coupling is limited. The lactate concentration is close to the detection limit (1 mmol) and the other metabolites (glucose, GABA, glutamate, etc.) overlap each other and simple analysis based on J coupling is impossible.

On the other hand, there exist a group of *editing techniques* which enable identifying coupled nuclei – details see [13]. One of the simplest techniques is the observation of the evolution of the signal as the function of echo time. The phase of the CH_3 doublet of lactate in spin-echo sequence with $TE = 1/J = 144$ ms is negative to the phase at short (10–30 ms) or long echo time (270–290 ms) [16] and this phenomenon can be used for the interpretation of MR spectra (Fig. 4).

2.3. Signal intensity

As in other spectroscopic techniques, signal intensity is another basic parameter in the MR spectrum. Signal intensity in MR spectroscopy depends on many factors. In the case of in vivo MR spectroscopy a simplified general term can be written as

$$I_s \sim A(B_0, T)B(n, T1, T2)C(n_{acq}, \mathrm{VOI}, t, B_1) \tag{4}$$

where A represents parameters that are constant for all examinations such as the multiplicity of the measured nucleus, static magnetic field (B_0) and temperature (T). The term B describes the influence of the nuclei parameters – mainly the relaxation times ($T1$) and ($T2$), and the number of resonating nuclei n. Instrumental parameters which we can change in the experiment are included in term C (number of acquisitions n_{acq} or total time of measurement t, measured volume VOI, all parameters related to B_1 – receiver gain, flip angle, etc.) for more details see [12].

When we are measuring in vivo ^{1}H MR spectra with a routine MR imager we have to take into consideration only a few parameters which can be controlled by the pulse sequence to get the best signal-to-noise ratio (S/N) – Fig. 3. The S/N is the number which tells how strong is signal compared to the noise. The value of S/N can be obtained simply by the measurement of height of the noise (peak to peak) and the height of the signal. It can also be measured statistically. The S/N ratios together with half-width of the signal are the most important parameters characterizing the quality of measured spectra. Because of the low sensitivity of MR spectroscopy we use the time averaging technique. In this case the signal to noise ratio is increased proportionally with the square of the number of repetitions of pulse sequences – acquisitions.

$$\left(\frac{\mathrm{S}}{\mathrm{N}}\right)_n = \left(\frac{\mathrm{S}}{\mathrm{N}}\right)_{n=1} \sqrt{(n)} \tag{5}$$

The acquisition is the time necessary for the measurement of one single spectrum. The number of acquisitions is limited by the time which is available for the examination; nevertheless, under normal clinical conditions we have about 1 h for the whole examination (usually suitable for four to six spectra measurements) due to physiological and ethical reasons.

Another possibility to increase S/N is an increase in the size of VOI. This is useful when diffuse diseases are studied, but the line width of signals is the limiting factor because overall B_0 homogeneity in the large VOI is reduced compared with a small one. The signal to noise ratio strongly depends on the choice of parameters dependent on relaxation times $T1$ and $T2$ of measured metabolites. Relaxation time $T1$ describes the rate of the return of all nuclei under measurement to the equilibrium state. When we need spectra where signals are actually proportional to the number of nuclei, the repetition time should be at least five times longer than the longest $T1$ of the nuclei in the metabolite being measured.

Because the majority of sequences used in routine imagers are based on spin echo techniques, relaxation time $T2$ is a crucial parameter which influences signal intensity. For technical

reasons it is difficult to measure the signal just after excitation and a short ms delay is necessary. Due to the *T*2 effect there is a substantial decrease in the signal intensity of molecules with short *T*2 relaxation time – these are mainly the signals of lipids, etc. Conversely, this technique can be used for the simplification of spectra. Spectra of the brain measured with long echo time do not contain broad signals of lipids and can be more easily quantitatively interpreted (Fig. 5). Details on quantitation are described in the paper by Helms [12].

3. Metabolites measured by in vivo MR spectroscopy

The volume of interest contains four types of compounds interesting from the point of view of in vivo MR spectroscopy (Fig. 5):

1) water with a concentration of about 40 M (in the brain);
2) macromolecules, lipids;
3) metabolites with molecular weight below 2000 Da and concentration >1 mM;
4) metabolites with molecular weight of about 2000 Da and concentration <1 mM.

The most important group in clinical application of ^{1}H MR spectroscopy is group 3 which represents about 20 compounds found in ^{1}H spectra of human tissue (Fig. 6). This group of compounds is visible very well in MR spectra and can be interpreted. Group 4 represents metabolites which can, in principle, be measured, but because of the limitation of experimental time, sufficient signal to noise cannot be obtained during a session. Compounds in group 2 produce broad signal or are invisible in the MR spectra. The water signal is important; nevertheless, the signal needs to be suppressed because its intensity is approximately four orders greater than the signal intensity of other visible metabolites. A list of chemical shifts of prominent metabolites is shown in Table 3.

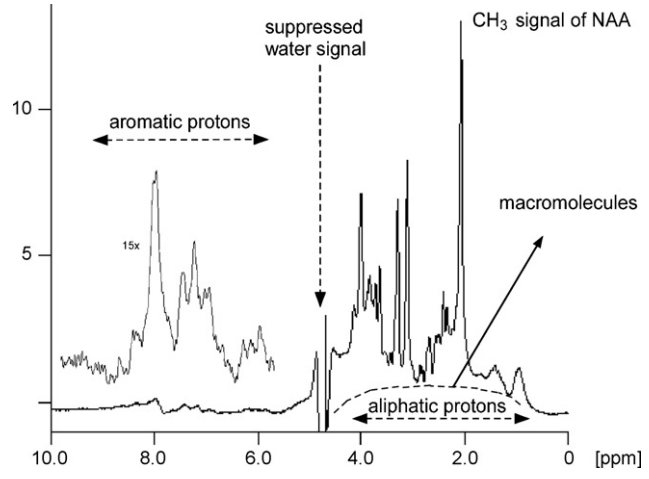

Fig. 5. Whole ^{1}H MR spectrum of human brain tissue obtained using a spin-echo sequence (TE = 30 ms, TR = 5000 ms, VOI = 28 ml, 3 T). In the region of aliphatic protons metabolite signals with concentrations higher than 1 mM are well visible and are positioned above broad signal of macromolecules. The water signal is suppressed, nevertheless, the range of approximately 4.3–5.1 ppm is influenced by many artifacts and cannot be used for the inspection of the spectrum. The aromatic part of the spectrum contains signals of compounds with very low concentrations (in normal brains) such as phenylalanine, histidines, histamines, etc.

Changes in genes induce changes in biochemical pathways. The result of these changes can be interpreted by using a metabolic profile which represents the set of molecules (molecular weight <2000 Da) observable by various analytical methods.

It is interesting that from millions of compounds acting in the human body only about 3000 metabolites are precursors or end-products of metabolic pathways and can be well analyzed by present analytical methods. The results of such methods can be used for the description of the biochemical status of a patient. The discipline, which studies these metabolites, is known as metabolic profiling (metabolomics or metabonomics) and useful analytical methods are mass spectrometry, high resolution NMR, chromatography and others.

With in vivo ^{1}H MR spectroscopy as was mentioned previously, we can currently observe only approximately 20 metabolites, this means that the metabolic profile is low in information. This is only a small part of all metabolites which can be found changed in the human body in diseases. Despite such a low number of observable metabolites, MR spectroscopy in vivo provides huge information about metabolism inside living tissue without invasive intervention. Among other metabolites, three major representatives characterize typical brain ^{1}H MR spectrum – *N*-acetylaspartate, creatine/phosphocreatine and choline compounds (Fig. 7).

3.1. N-acetylaspartate

N-acetylaspartate (NAA) was first described in 1956 by Tallan [17,18] who observed neuronal cells of various vertebrates but almost no attention were paid to this compound for the next 40 years. Its exact role as a metabolite is still almost unknown and discussed [19].

In accordance with recent findings, NAA synthesis is located in neuronal mitochondria and it needs L-aspartate-*N*-acetyltransferase (Asp-NAT EC 2.3.1.17) which is a membrane bound enzyme detectable only in the nervous system and is highly specific to aspartate. Aspartate thus can react with acetyl-CoA resulting in NAA production. This reaction proceeds when two other demands for acetyl-CoA have been met. They are (1) neuronal lipid synthesis via the ATP-citric lyase pathway and (2) energy production via the Krebs cycle.

NAA is then transported out of the mitochondria and out of neurons into oligodendrocytes probably using specific transporters. In oligodendrocytes NAA is degraded to aspartate and free acetate using aspartoacylase (ASPA, EC 3.5.1.15).

NAA creates the most prominent signal in ^{1}H MR spectra of the human brain (CH_3 group resonating at 2 ppm), and is considered to be a neuronal marker because it is located almost exclusively in neurons. It is more concentrated in gray matter than in white matter. Its concentration correlates well with myelination during brain maturation [20], and in the adult brain it reaches about 10–12 mM.

The signal observed in ^{1}H MR spectra comes not only from *N*-acetylaspartate itself (>80%), but *N*-acetylaspartylglutamate

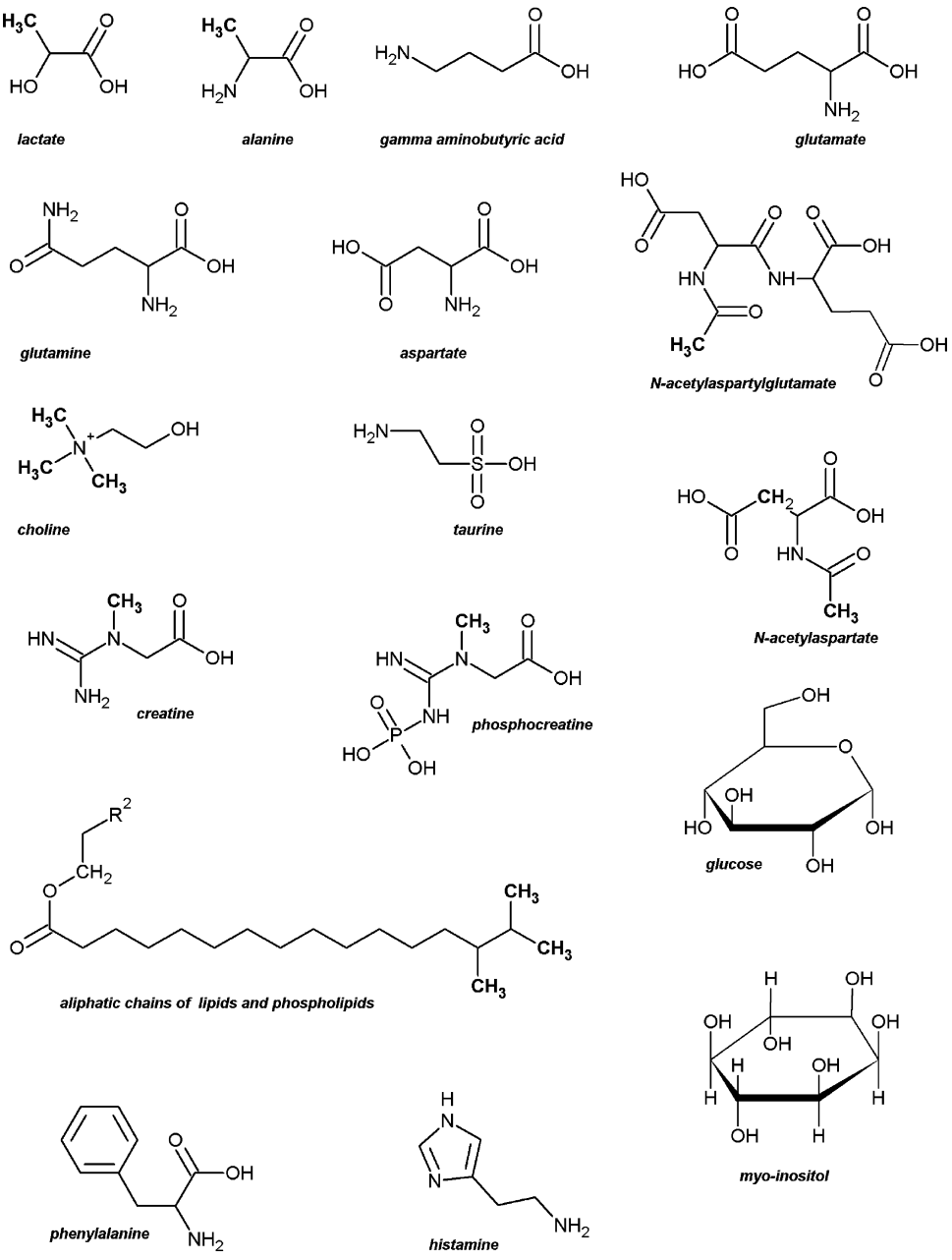

Fig. 6. Chemical structures of selected metabolites visible in ^{1}H MR spectra in the brain in vivo.

(NAAG), *N*-acetylglutamate and *N*-acetylglucosamine also contribute. In the basal ganglia or the posterior fossa NAAG can represent up to 20% of the total NAA signal. NAAG is the form in which the neuron stores glutamate to protect the cell from its effects.

3.2. Creatine/phosphocreatine

Creatine (Cr) together with phosphocreatine (PCr) are widely known as metabolites necessary for energetic supply for biochemical processes. In MR spectroscopy in vivo, their signals are clearly observed in proton (^{1}H) as well as in phosphorus (^{31}P) MR spectra and are often considered to be a marker of energetic metabolism.

Because of the similarity of Cr and PCr as chemical compounds, proton resonances of their CH_3 (3.01 ppm) and CH_2 (3.9 ppm) groups cannot be distinguished with standard clinical MR units (up to 7 T) and their sum is thus mentioned. Additional information about creatine metabolism can be obtained from ^{31}P MR spectroscopy, where, with the exception of the liver, the signal of PCr is dominant (mainly in muscles).

Besides creatine uptake from diet, about 50% of the total creatine amount is synthesized endogenously which consists of two steps. First, an enzyme arginine-glycine amidinotransferase (AGAT, EC 2.1.4.1) enables guanidino group transfer from arginine to glycine resulting in guanidinoacetate (GAA) and ornithine. GAA is then methylated by guanidinoacetate methyltransferase (GAMT, EC 2.1.1.2) to creatine. The crea-

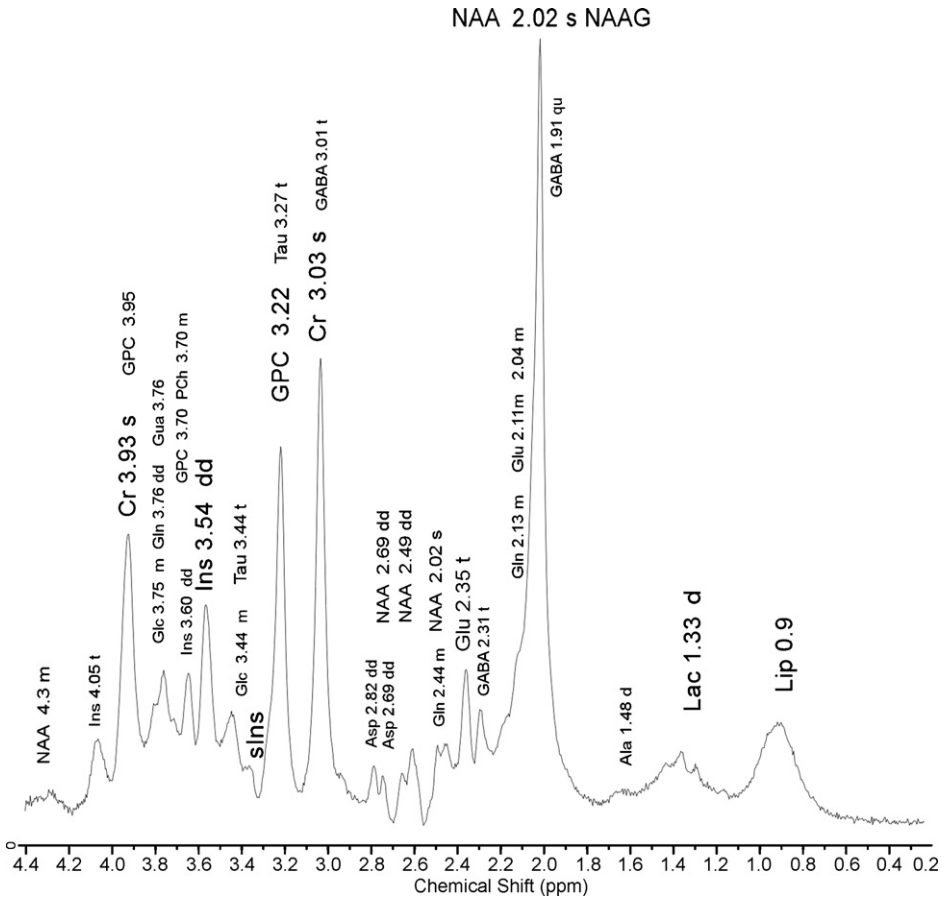

Fig. 7. The interpretation of the typical ^{1}H MR aliphatic part of the spectrum from Fig. 5 after phase, base line correction and elimination of macromolecule signals using LCModel software. NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartylglutamate; Cr/PCr, creatine/phosphocreatine; Cho, choline-containing compounds; GPC, glycerophosphocholine; Pch, phosphocholine; Ins, myo-inositol; sins, scyllo-inositol; Glc, glucose; Gln, glutamine; Glu, glutamate; Tau, taurine; Lip, lipids; Lac, lactate; Ala, alanine; GABA, γ butyric acid; Asp, aspartate (s, singlet; d, doublet; t, triplet; dd, doublet of doublet; m, multiplet).

tine circulates in the blood and enters the cells (primarily in muscles and kidneys, but also in the CNS and other organs) via an active transmembrane creatine transporter (CRTR). Creatine and phosphocreatine are later metabolized to creatinine which is excreted by the kidneys in urine.

3.3. Choline compounds

An intense signal in the brain spectrum resonating at 3.2 ppm belongs to choline containing compounds (Cho). The main contribution to this signal is produced by phosphocholine (PCho) and glycerophosphocholine (GPC). This signal is also contributed to by free cholines, citidine diphosphate choline (CDP-Cho), acetylcholine, betaine and others. As the MR visible signal only reflects water soluble choline metabolites, sphingomyelin and phosphatidylcholine (PtdCho, lecithin), that form a relatively immobile membrane component, do not contribute to the total signal intensity.

In ^{1}H MR spectroscopy the Cho signal can be considered a marker of membranes, because it incorporates precursors or degradation products of the membrane phospholipids. Its increasing intensity well reflects, for example, membrane disruption, cell proliferation (e.g. brain tumors [21,22]) or inflammation by macrophages [23]. Except for early childhood [20] the total concentration calculated from this signal is stable and its changes become a very sensitive marker to various diseases. It is necessary to take into account different signal intensity in different parts of the brain. It is known that the concentration in astrocytes is twice that in neurons; a greater choline signal can be found in white matter than in gray matter.

The main biochemical pathways of free cholines are connected with synthesis of acetylcholine and PtdCho. Whereas production of acetylcholine takes place only inside cholinergic neurons, all cells transform choline to PCho, which is further used via CDP-Cho for the synthesis of PtdCho. PtdCho is later degradated to GPC using phospholipase A_1 or A_2 (PLA_1 and PLA_2). Recently, it is believed that the PLA_2 enzyme plays a major role in the regulation of the intensity of the choline signal in MR spectra [24].

Several metabolites visible in MR spectra have relatively high concentrations but due to the multiplicity, their signal intensities are small and superpose each other (e.g. glutamate, glutamine, GABA, and myo-inositol). Some other metabolites are present in normal tissue, however, in very low concentration, i.e. too low signal intensity (e.g. alanine, lactate, etc.). Additional information about them can be found in other papers in this issue.

4. Conclusion

In other papers in this issue the reader can find basic information on how in vivo MR spectra can be measured and examples of the successful use of the results of proton spectroscopy for various clinical examinations and research studies.

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