MAGNETIC RESONANCE IN MEDICINE

Determination of Saturation Factors in ^{31}P NMR Spectra of the Developing Human Brain

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In order to assess the influence of longitudinal relaxation on previously reported variations in ^{31}P NMR signals during brain development, we used an accelerated two-point technique to determine T_1 at 2.35 Tesla in 8 min. Comparison between 10 normal neonates (age range 37–46 weeks postconception) and 10 healthy infants (age range 80–157 weeks postconception) indicated that T_1 does not vary substantially during the first year of life, except in the phosphomonoester (PME) region of the spectra. T_1 of total PME decreases with age which we could explain by its variable multicomponent nature: The signal from (unresolved) components at the downfield shoulder of PME (attributed mostly to phosphorylethanolamine at 6.72 ppm) with a T_1 of at least 6.4 s decreases with age relative to contributions from other phosphomonoester compounds resonating predominantly at the upfield side of the peak (approximately 6.3 ppm), with T_1 below 2.9 s. Because the T_1 heterogeneity of PME may depend on its relative composition, quantitative ^{31}P NMR spectroscopy may require an assessment of the influence of longitudinal relaxation on the signal amplitudes in each measurement.

INTRODUCTION

Age-related changes in the ^{31}P NMR spectra obtained from the human postnatal brain have been characterized in a previous study using surface coils (1). As in most other studies of the developing human brain (2, 3), the experiments were performed with repetition times of the order of the longitudinal relaxation times T_1 and signal intensities were influenced by incomplete T_1 relaxation between successive acquisitions. An accurate quantitation of the resonances may be severely affected when T_1 varies during brain development. In addition, the time course of relative concentrations may well be significantly altered.

The acquisition of spectra unaffected by saturation effects is possible when the repetition time is at least three times longer than the maximal T_1 present in the spectrum. However, this is clearly not practical *in vivo* in terms of optimal signal-to-noise.

It was the purpose of this study to assess changes in saturation effects in the ^{31}P NMR spectra during brain development. Since from a comparison of different studies a change in the T_1 for the phosphomonoester (PME) region with development could be inferred (2, 4, 5), we also studied changes in the PME region during the 1st year of life using surface coil and volume selective techniques.

Since the study involved uncooperative infants that had to be examined during sleep, time-consuming and motion-sensitive techniques such as inversion recovery were clearly not applicable. Instead we were forced to use a previously described technique for the measurement of T_1, which acquires the necessary data simultaneously and requires a maximal experimental time of only 8 min using a 5.5-cm diameter surface coil (6). This represents a substantial advantage over the 45 to 60 min typically needed for motion-sensitive methods that require the acquisition of fully relaxed spectra, such as inversion recovery experiments obtained from 500-ml volumes (4, 5).

METHODS

Patients

The study included 10 neonates in Group 1 with the age ranging from 37 to 46 postconceptional weeks (median = 41 weeks). Group 2 included 10 infants ranging in age from 80 to 157 weeks postconception with a median of 99 weeks. In Group 1 the measurement was performed during postprandial sleep, whereas the infants in Group 2 had to be sedated with chloralhydrate (100 mg/kg, Dentinox, Berlin, Germany). All children were neurologically normal. Fully informed parental consent was obtained according to forms and procedures approved by the local Ethical committee.

T_1 Measurements

The children were positioned on a double-tuned $^{1}H/^{31}P$ surface coil (5.5-cm diameter). The signal was recorded predominantly from the occipital region. All measurements were performed in a 2.35 T magnet (MEDSPEC 24/40 Bruker-Spectrospin AG, Fällanden, Switzerland) with 35 cm of clear access (7).

Contamination of the ^{31}P NMR brain spectra with signal from superficial muscle tissue was minimized by placing a ferromagnetically dotted sheet (spoiler) between surface coil and head which efficiently suppressed signals from the surface by selectively spoiling the static field homogeneity (8). The position and the effect of the spoiler were monitored in images that were acquired in all three orthogonal magnet axes using gradient-recalled echoes (echo time TE = 15 ms, 5-mm slice thickness, 20-cm field of view, TR = 60 ms).

Since the study was performed with mildly sedated infants and neonates, we had to devise a rapid method to measure T_1 with surface coils, which has been described in detail in Ref. 6. We showed in human calf muscle the

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reliability and efficiency of the *in vivo* T_1 measurements using the pulse sequence depicted in Fig. 1. To assess sequence performance, additional measurements with different interpulse delays (τ in Fig. 1) were thereby important, since in the ideal case of monoexponential relaxation, calculated T_1 values are independent of τ. On the other hand, any deviation of the pulses from 90° and 180°, respectively, will result in a T_1 depending on this experimental parameter. We showed in particular that T_1 of the most intense peak in muscle, namely phosphocreatine (PCr), was independent of the interpulse delay τ, indicating accurate T_1 values. As part of the testing procedures, we applied the method to several different phantoms using ^{31}P and ^{13}C NMR. These measurements showed that the resulting T_1 varied by less than 10% when τ was between $T_1/3$ and $2T_1$. In one of these phantoms, which contained AMP and PO_4, the measurements using the sequence in Fig. 1 were compared with an inversion recovery (IR) experiment performed in a resonator (9). The homogeneous B_1 allowed to use hard pulses that were applied on resonance. For both compounds, the T_1 values obtained with the sequence in Fig. 1 using the 5.5-cm surface coil were within 10% of those measured with the IR experiment in the resonator.

Positioning, tuning, and shimming required at most 10 min. The first T_1 measurement was obtained by using the sequence of Fig. 1 with an interpulse delay $\tau = 2.65$ s and collecting 64 scans in each memory block S1 and S2, respectively, which resulted in an experimental time of 8 min. The minimal time required for a baby to stay in the magnet was thus 15 min. In 19 children we were able to add another 8-min measurement with $\tau = 5.15$ s (32 scans).

Time-domain signals (acquisition time was 123 ms) were multiplied with a trapezoidal function (0, 1, 50, 50 ms) and zero-filled to 500 ms prior to Fourier transformation (FT). The residual broad component underlying the brain spectra was eliminated by baseline correcting the region from 20 to −30 ppm with a fourth-order polynomial. From the signals S1 and S2 obtained with the sequence of Fig. 1, we determined the ratio $\lambda = S2/S1$ for each individual line by minimizing the difference spectrum S2-λS1, using standard software (Bruker, Karlsruhe, Germany). This procedure proved to be little sensitive to a non-zero baseline (6).

The chemical shift of resonances is given relative to PCr set to 0 ppm, i.e., PME at 6.7 ppm, inorganic phosphate (P_i) at 4.9 ppm, phosphodiester resonances (PDE): 2 to 4 ppm, γ-, α-, and β-phosphate of nucleotide triphosphates (NTP) at −2.4 ppm, −7.6 ppm, and −16.2 ppm, respectively.

Volume Selective Spectroscopy

Gradient-localized spectroscopy was used to achieve reproducible line widths in well-defined volumes localized in the center of the head using a home-built double-tuned resonator (9). A 64-ml volume was localized in the central nuclei comprising basal ganglia and thalamus using an eddy-current compensated ISIS sequence (10) with the same inversion pulses as the one used in Fig. 1, which produced a 3.3-kHz inversion bandwidth (6). The

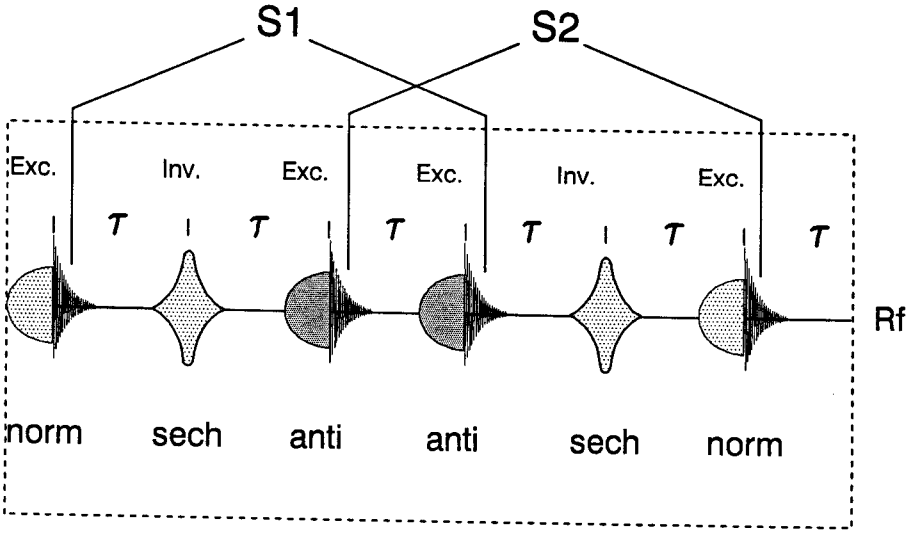

FIG. 1. *Pulse sequence used to determine the T_1 values of ^{31}P NMR spectra.* The signals S1 and S2 are acquired at steady state and stored in separate memory blocks. The excitation pulses are designed to sample the *z* magnetization and transverse coherence between pulses is destroyed by the short T_2 of phosphorus *in vivo*. T_1 was calculated from S2/S1 and the interpulse delay τ as

$$T_1 = -\frac{\tau}{\ln(1 - S2/S1)} \quad [1]$$

6 ms hyperbolic secant pulses (17) were used for *inversion* (denoted by "sech"). 3 ms adiabatic half passage pulses of the numerically optimized sin/cos type (18, 19) that sweep from opposite sides of the spectrum onto resonance (denoted by "norm" or "anti," respectively) were used for *excitation*, as described in Ref. 6.

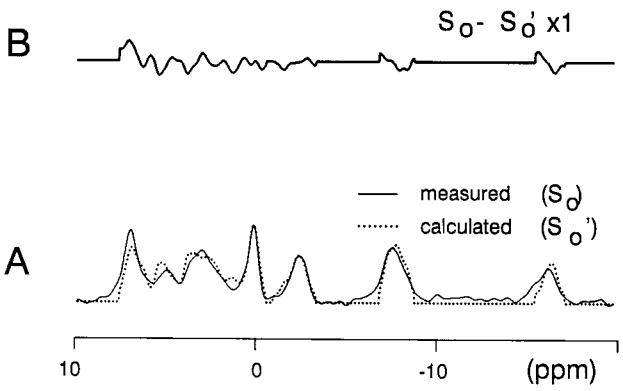

FIG. 2. *Comparison of a fully relaxed spectrum, S_0, with a spectrum extrapolated from the T_1 measurement, S_0'.* (A) The spectrum S_0 was acquired under fully relaxed conditions (TR = 35 s, 32 scans, 16-min experimental time) using the same excitation pulses as in Fig. 1. The spectrum S_0' was calculated point-by-point from the spectra S1 and S2 obtained with the sequence of Fig. 1 (τ = 2.65 s, 64 scans, 8 min). (B) shows the differences between both spectra. Only those points were used whose signal was well above the noise level in both spectra S1 and S2 and whose T_1 values were positive, otherwise S_0', as well as S_0-S_0', was set to zero.

excitation pulse was a 250-μs rectangular pulse whose power was adjusted according to a calibrated scale attached to the matching capacitor (11, 12). Automated localized shimming with the X, Y, Z, Z^2 and X^2-Y^2 coils was used (13) resulting in reproducible line widths between 6 and 8 Hz for PCr. Nine healthy children (four corresponding to Group 1 and five in Group 2 above) were studied after sedation with chloral hydrate (100 mg/kg, Dentinox, Berlin, Germany).

RESULTS

The reliability of the sequence in Fig. 1 was tested in the brain of three children. The fully relaxed spectrum S_0, was compared to the spectrum S_0', calculated from the spectra S1 and S2 that were obtained from the saturation factor measurement (Fig. 2A) using the same adiabatic half passage pulses for excitation. The differences (Fig. 2B) are within the signal-to-noise of the involved measurements, indicating very good performance of the method also in human brain.

The T_1 measurements obtained with an interpulse delay of τ = 2.65 s (Fig. 1) are summarized for both age groups in Table 1A. The differences between the two groups are statistically significant only for PME ($P < 0.01$) and PCr ($P < 0.05$). In 19 subjects, we were able to add a control measurement where the interpulse delay τ was set to τ = 5.15 s (Fig. 1). The resulting T_1 measurements (Table 1B) show that T_1 of PME varies significantly between the two groups ($P < 0.05$) as in the measurement obtained with τ = 2.65 s. T_1 is approximately independent of τ for PCr, as can be seen from Table 1, whereas for P_i, PDE, and the NTP resonances, T_1 as well as its relative error increased with the experimental parameter τ of Fig. 1.

Based on the T_1 measurements presented in Table 1, we calculated the corresponding saturation factors (SF) for a standard pulse-and-acquire experiment with a repetition time TR of 3 s and assuming perfect 90° excitation (Table 2). All saturation factors in Table 2 are fairly constant with the notable exception of PME whose saturation factor varies substantially with age.

TABLE 1
T_1 in s (mean ± SD) Measured in 10 Neonates (Group 1) and in 10 Infants (Group 2)

	PME	P_i	PDE	PCr	γNTP	αNTP	βNTP
(A) T_1 measurements (s) performed with τ = 2.65 s (Fig. 1)							
Group 1	4.9 ± 1.0	1.8 ± 0.6	1.7 ± 0.4	4.4 ± 1.2	1.3 ± 0.3	1.5 ± 0.4	1.6 ± 0.5
Group 2	2.9 ± 0.6	1.8 ± 0.5	1.6 ± 0.2	3.2 ± 0.3	1.5 ± 0.3	1.7 ± 0.3	1.3 ± 0.4
Difference[a]	−2.0 ± 0.4	0.0 ± 0.25	−0.1 ± 0.15	−1.2 ± 0.9	0.2 ± 0.1	0.2 ± 0.15	−0.3 ± 0.2
(B) T_1 measurements (s) at τ = 5.15 s (Fig. 1).[b]							
Group 1[c]	6.4 ± 1.0	3.4 ± 1.1	2.1 ± 0.7	3.9 ± 1.0	2.2 ± 0.6	2.7 ± 1.0	1.7 ± 0.6[d]
Group 2	4.9 ± 0.6	2.2 ± 0.6	2.0 ± 0.6	3.7 ± 1.1	2.2 ± 0.6	2.3 ± 0.8	2.5 ± 0.7
Difference[a]	−1.6 ± 0.45	−1.2 ± 0.45	−0.2 ± 0.35	−0.2 ± 0.65	−0.1 ± 0.4	−0.6 ± 0.6	0.8 ± 0.35

[a] Given is mean ± standard error (in s) based on a paired *t* test.
[b] The measurements at τ = 5.15 s were also performed in 8 min, resulting in a substantially increased relative error than the measurements with τ = 2.65 s (Table 1A).
[c] Only nine neonates could be measured with τ = 5.15 s.
[d] Measurement of T_1 of βNTP was only possible in six neonates at τ = 5.15 s due to impaired signal-to-noise.

TABLE 2
The Saturation Factors at a TR = 3 s for Homogeneous 90° Pulses[a]

	PME	P_i	PDE	PCr	γNTP	αNTP	βNTP
Group 1	2.2(2.7)[b]	1.2(1.7)	1.2(1.3)	2.0(1.9)	1.1(1.3)	1.2(1.5)	1.2(1.2)
Group 1	1.6(2.2)	1.2(1.3)	1.2(1.3)	1.6(1.8)	1.2(1.3)	1.2(1.4)	1.1(1.4)

[a] We define the saturation factor (SF) to be that number by which the integral has to be multiplied to give the unsaturated intensity, hence SF ≡ $(1-\exp(-TR/T_1))^{-1}$.
[b] All numbers are calculated from Table 1A, in parentheses from Table 1B.

For a homogeneous resonance with a single exponential relaxation behavior, the signal in S2 (Fig. 1) should be equal to that in S1 multiplied by a constant factor, and the measured T_1 should be independent of the experimental parameter τ, as is the case for PCr (Table 1). However, this is especially not true for PME, where T_1 increased with τ (Tables 1A and 1B). Additionally, we observed in at least 70% of the measurements that the line-width of PME increased in S2 with an additional upfield shoulder, in contrast to all other resonances. These findings are illustrated in Fig. 3A, where spectra S1 and S2 that were recorded with the sequence of Fig. 1 are shown for a neonate (Fig. 3A, bottom) as well as for a 15-month-old infant (top). In 85% of the measurements, a point-by-point calculation of T_1 indicated a higher T_1 at the downfield shoulder of PME. This can also be seen from Fig. 3B, where the spectra S2 scaled to match the height of S1 at 6.7 ppm are in both age groups higher than S1 at 6.3 ppm (shaded area), whereas S2 should equal S1 if PME had a homogeneous T_1. The T_1 difference was less for the Group 2 children.

The line widths of the spectra obtained in this study using a surface coil prohibited the separation of the individual components of PME. We have therefore acquired gradient-localized spectra from the central nuclei of nine additional children in order to resolve the substructure of the PME peak. The line width of PCr was always between 6 and 8 Hz in these measurements. The results showed consistently a vanishing component at the down-field shoulder of PME (arrow in Fig. 4), resulting in an *apparent* broadening of PME with increasing age.

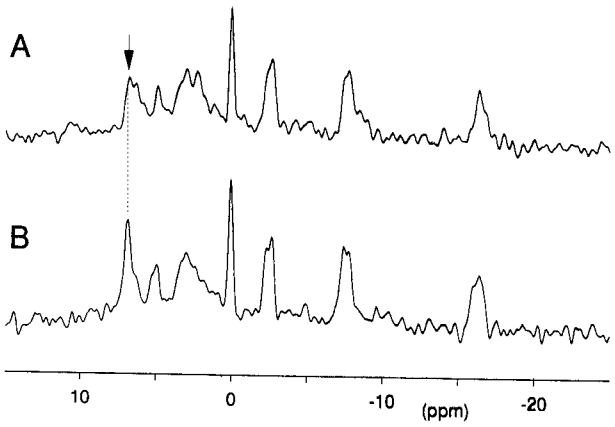

FIG. 4 *Gradient-localized spectra in the central nuclei of two healthy infants at 9 months (A) and at birth (B).* The spectra were acquired with a modified ISIS-Sequence (10, 11) from a 64-ml volume. 1536 scans were collected and the repetition time TR was 2.7 s. The signals were multiplied with a 5-Hz exponential multiplication prior to FT and are shown without baseline correction. Note the increase of the apparent line width of PME in the older infant (B) which is due to a decreased component at 6.7 ppm (arrow) relative to the signal intensity at 6.2 ppm.

DISCUSSION

We conclude from Table 1 that the variation of the signal ratios PCr/βNTP and αNTP/βNTP reported earlier (1) are unaffected by changes in T_1 and, therefore, represent relative concentration changes, whereas [PME]/[PDE] decays more rapidly with age than the signal ratio PME/PDE due to the decreasing T_1 of PME.

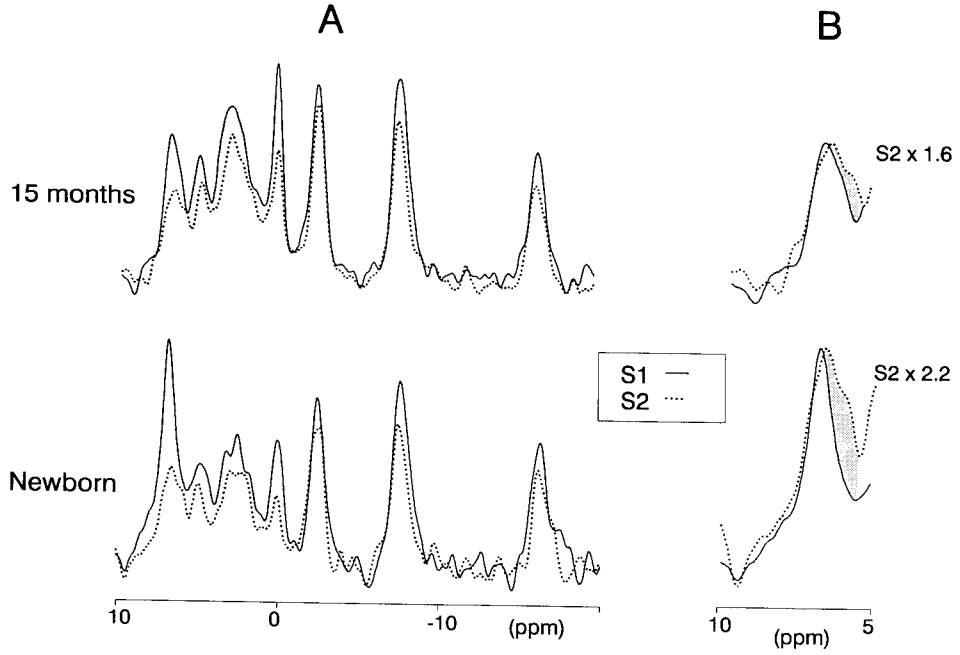

FIG. 3. *Sample spectra S1 and S2 obtained in the neonates (Group 1) and in the infants (Group 2).* (A) The bottom measurement is from a newborn (41 weeks postconceptional age) whereas the top is from an infant (99 weeks). (B) Expansion of the PME region of (A). S2 (dotted line) was multiplied by a constant factor (as indicated) to match the height of PME in S1 (solid line) at 6.7 ppm. With this scaling, S2 is at the upfield side of PME (~6.3 ppm) higher than S1 (shaded area) indicating faster relaxation than at 6.7 ppm. 64 averages were collected and τ was 2.65 s resulting in 8-min experimental time. Processing was as described in Methods section.

The PME resonance is the only peak in the spectra whose T_1 shows a consistent decrease with age as can be seen from Table 1A and 1B. The observation that the PME peak broadens under conditions of increased saturation (S2 in Fig. 3) suggests that PME is a composite peak and its components have different T_1 as well as different unresolved chemical shifts. A variable relative content may therefore result in a change in the apparent T_1 of the total PME peak.

For a composite resonance with two different longitudinal relaxation times T_{1a} and T_{1b}, the two-point measurement used here and shown in Fig. 1 will give T_1 values that depend on the experimental parameter τ (6). The resulting T_1 value, however, will always lie between the two extremes, T_{1a} and T_{1b}. The obtained value depends on the relative concentration of the two components and the particular choice of τ. We therefore conclude from Table 1B that the slowly relaxing component has a T_1 of at least 6.4 s and from Table 1A that the T_1 of the faster relaxing compartment is below 2.9 s.

It has been shown in earlier studies that the dominant constituent of PME in the dog puppy brain is phosphorylethanolamine (PEt) (14). Another study correlated the decrease of PME in the rat brain with the concentration of PEt measured in brain extracts (15). Our observations suggest that the PME peak has at least two different components with one signal (possibly PEt) relaxing very slowly compared with the other constituents. PEt has under normal physiological conditions a chemical shift of 6.72 ppm which is down field from, e.g., phosphocholine (PCho, 6.24 ppm) and other phosphomonoesters (14, 16), which provides further evidence indicating that PEt has a long T_1 *in vivo*.

The line width of PME at half-height also increases during brain development, as shown in Fig. 4 using gradient-localized spectroscopy, which indicates that the *relative* contribution of a single resonance decreases *in vivo* with development. We suggest that this resonance, which dominates at birth, can be attributed mostly to PEt and has a long T_1 *in vivo*.

The present study may provide an explanation of the high saturation factor measured for PME in the *newborn* human brain, corresponding to a T_1 above 5 s (2) and the relatively short T_1 (below 3 s) measured in spectra of the *adult* human brain (4, 5). We conclude that if PEt dominates the PME peak in the ^{31}P NMR spectra of the newborn brain, it has a long T_1 (>6.4 s), whereas multiple unresolved resonances with short T_1 (<2.9 s) contribute mostly to the PME peak in spectra of the adult brain.

Alternatively, it is in principle possible that T_1 of PEt may change with age, but this is difficult to prove experimentally due to the overlap with other resonances. In contrast, this study shows that T_1 of PME is heterogeneous (Fig. 3) and that the signal intensity at 6.2 ppm is approximately constant relative to NTP (Fig. 4). Since most changes in the PME peak have been shown to occur in the 1st postpartal year (1, 3), it is therefore most likely that the decrease in the slowly relaxing component is the main cause for the drop in apparent T_1 of the total PME peak.

The variability of the PME resonance in relative composition and therefore in T_1 emphasizes the need for an assessment of saturation effects in each measurement. This might also be true in liver or tumor spectroscopy where PME variations have been reported (20–22). However, changes in PME heterogeneity may have been obscured, since the very good separation of the Pi peak from neighboring resonances in Fig. 4 indicates a considerable improvement in spectral resolution compared to these studies.

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