A ³¹Phosphorous Magnetic Resonance Spectroscopy Study of Diazepam Does Not Affect Brain Phosphorous Metabolism

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Introduction

We utilized in vivo ³¹Phosphorous magnetic resonance spectroscopy (³¹P MRS) to determine whether acute administration of diazepam alters brain oxidative and phospholipid metabolism in normal subjects. A primary motivation for these experiments was to establish whether sedation with diazepam, which is often required for patients to be successfully studied with MRS, alters ³¹P metabolites. We present here the results of an initial study of eight subjects using 10 mg of diazepam and a second study of ten subjects using 20 mg of diazepam.

Methods

Eight men who were volunteers (mean ± SD = 28.4 ± 4.8 years) participated in the 10 mg study, and 9 men and 1 woman (mean ± SD ¬ 27.8 ± 4.4 years) participated in the 20 mg

study. One subject participated in both studies. All subjects were free of medical, neurological, or psychiatric problems and denied a history of alcohol or substance abuse. None were taking any medication at the time of the study. All subjects underwent a control ³¹P MRS study in the fasting state, followed by their dosage of oral diazepam and a repeat ³¹P MRS study 1 hr later.

All studies were performed on a Philips Gyroscan 2.0 Tesla S15 MRI/MRS system, operating at 34.79 MHz for ³¹Phosphorus. Subjects underwent T_1-weighted (TR = 600 msec; TE = 30 msec) sagittal and multislice axial images on which two spectroscopy volumes of interest (VOIs) were determined (see Figure 1). The first, (Region A, 127 ml, 8.5 × 6 × 2.5 cm³) consisted mainly of white matter, and was bordered inferiorly by the inferior margin of the corpus callosum and by the inner margin of the cortical mantle. The second, (Region B, 90 ml, 5 × 6 × 3 cm³) consisted primarily of subcortical gray matter structures with minimal white matter from the extreme, external, and internal capsules. This region was bounded anteriorly–inferiorly by sphenoid bone; third and inferior lateral ventricle cerebrospinal fluid (CSF) was included in this volume.

Using the improved ISIS sequence (Matson et al 1988), ³¹P spectra of the VOI were acquired
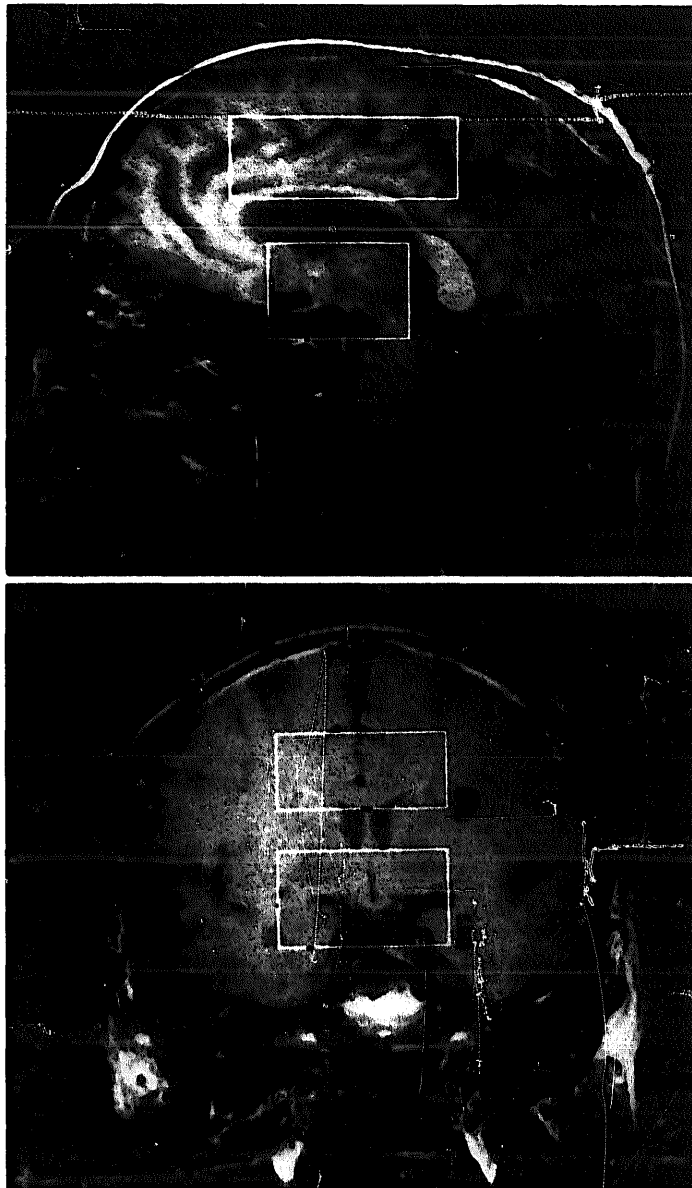
Figure 1. Top: midsagittal MRI showing region A (1) and region B (2). Bottom: midcoronal MRI showing region A (1) and region B (2).

with a 16.5 cm diameter Helmholtz head coil for 640 scans with a repetition time of 2 sec. Absolute molar concentrations were estimated as previously described (Roth et al 1989; Lawry et al 1989; Deicken et al 1991).

Statistical Analysis:

To test for changes due to diazepam, the Wilcoxon test was applied to metabolite concentrations, ratios of concentrations, and pH, sep-arately for data from the 10 mg and 20 mg studies.

In the second stage of analysis, we combined the data from the two studies, increasing the precision of the statistical estimates. We first computed the postdiazepam to prediazepam ratio of each metabolite concentration and pH. We assumed a linear regression model of the form:

$$\log(R_{ij}) = \beta j + e_{ij}, \quad i = 1, \ldots, n_j,$$
$$j = 1, 2,$$

in which log denotes the natural logarithm, j is the dose of diazepam measured in tens of milligrams, ($e.g.$, $j = 2$ denotes a dose of 20 mg), β is a regression coefficient, and e_{ij} denotes random error. We assumed that the errors were independent and normally distributed, neglecting possible dependence between measurements on the one subject that appeared in both studies.

The model had no intercept, corresponding to an assumption of no effect of a zero dose. The data from the 10 mg study were considerably more variable than those in the 20 mg study, due to periodic Gyroscan preamplifier variation that was resolved before we undertook the 20 mg study. To account for this, we used weighted least squares (WLS) (Draper and Smith 1966).

We computed confidence intervals for the percentage change in [PCr] and [β-ATP], the two metabolite concentrations we judged to have the greatest potential clinical importance. We applied the Bonferroni correction based on computing four intervals (two metabolites by two brain regions).

Results

For both the 10 mg and 20 mg study (Table 1), the Wilcoxon tests revealed no significant differences in metabolite concentrations, ratios, or pH in either Region A or Region B as a consequence of diazepam administration.

Table 2 (top) gives the estimated percentage change caused by increasing the diazepam dose by 10 mg. All the change estimates in Table 2 are very close to zero, giving further evidence that diazepam has little or no effect on these metabolites and pH.

Table 2 (bottom) gives the confidence intervals for the percentage change in [PCr] and [β-ATP]. All confidence intervals include zero change. For Region A, the greatest change included within the intervals is less than 10%. For Region B, the interval for [β-ATP] includes a change of + 19.8%. Although these results indicate substantial statistical variability in the change estimates, they still indicate that the diazepam effect is quite moderate, if it exists at all.

Table 1. Comparison of ^{31}P Metabolites and pH in Subjects before and after 10 mg Diazepam ($n = 8$)[a]

Region A	No diazepam (mean ± SE mM)	Diazepam (mean ± SE mM)	Region B	Nodiazepam (mean ± SE mM)	Diazepam (mean ± SE mM)
[PME]	3.11 ± 0.31	3.08 ± 0.25	[PME]	2.37 ± 0.28	2.05 ± 0.17
[Pi]	1.34 ± 0.15	1.61 ± 0.18	[Pi]	1.67 ± 0.17	1.04 ± 0.13
[PDE]	8.98 ± 0.57	9.46 ± 0.68	[PDE]	6.10 ± 0.97	5.77 ± 0.50
[PCr]	3.37 ± 0.23	3.30 ± 0.26	[PCr]	2.82 ± 0.26	2.60 ± 0.18
[β-ATP]	1.77 ± 0.20	1.75 ± 0.16	[β-ATP]	1.15 ± 0.10	1.21 ± 0.16
[PCr]/[Pi]	2.77 ± 0.35	2.23 ± 0.33	[PCr]/[Pi]	2.75 ± 0.40	2.74 ± 0.28
[PCr]/[β-ATP]	2.04 ± 0.19	1.90 ± 0.54	[PCr]/[β-ATP]	2.48 ± 0.20	2.29 ± 0.27
[β-ATP]/[Pi]	1.42 ± 0.19	1.24 ± 0.20	[β-ATP]/[Pi]	1.15 ± 0.18	1.33 ± 0.26
pH	7.03 ± 0.04	7.01 ± 0.02	pH	7.06 ± 0.02	7.06 ± 0.03
Comparison of ^{31}P Metabolites and pH in Subjects before and after 20 mg Diazepam ($n = 10$)[a]					
[PME]	3.72 ± 0.21	3.51 ± 0.29	[PME]	3.28 ± 0.31	3.10 ± 0.14
[Pi]	1.50 ± 0.15	1.52 ± 0.14	[Pi]	1.56 ± 0.12	1.54 ± 0.10
[PDE]	10.20 ± 0.50	10.17 ± 0.72	[PDE]	7.73 ± 0.34	8.10 ± 0.34
[PCr]	3.82 ± 0.22	3.79 ± 0.24	[PCr]	4.03 ± 0.23	4.35 ± 0.26
[β-ATP]	2.17 ± 0.17	2.08 ± 0.15	[β-ATP]	1.63 ± 0.10	1.79 ± 0.25
[PCr]/[Pi]	2.73 ± 0.24	2.62 ± 0.24	[PCr]/[Pi]	2.64 ± 0.15	2.92 ± 0.22
[PCr]/[β-ATP]	1.81 ± 0.10	1.84 ± 0.06	[PCr]/[β-ATP]	2.58 ± 0.24	2.48 ± 0.14
[β-ATP]/[Pi]	1.51 ± 0.10	1.42 ± 0.07	[β-ATP]/[Pi]	1.63 ± 0.10	1.79 ± 0.25
pH	7.04 ± 0.01	7.04 ± 0.01	pH	7.09 ± 0.02	7.08 ± 0.01

[a]No significant differences by Wilcoxon signed rank test.

Table 2. Effects of Diazepam on Metabolite
Concentrations and pH in Regions A and B:
Estimates of Percentage Change Caused by
Increasing the Dose by 10 mg

	Region A	Region B
[PME]	−3.4	−2.7
[Pi]	+2.6	−0.8
[PDE]	+1.4	+2.5
[PCr]	−0.9	+3.3
[β-ATP]	−1.9	+4.8
pH	−0.1	−0.1

Bonferroni-Corrected 90% Confidence Intervals for the
  Percentage Change in [PCr] and [β-ATP] Caused by
  Increasing the Diazepam Dose by 10 mg

| [PCr] | (−8.2, +7.0) | (−3.2, +10.2) |
| [β-ATP] | (−5.9, +2.2) | (−8.3, +19.8) |

Discussion

Our study suggests that oral administration of a
single dose of diazepam up to 20 mg does not
significantly alter brain high energy phospho-
rous or phospholipid metabolism as detected by
in vivo ^{31}P MRS in either a primarily white
matter or a subcortical gray matter region. Thus,
diazepam can be utilized to sedate patients when
necessary for ^{31}P MRS studies without having
an appreciable effect on ^{31}P metabolites. To our
knowledge, this is the first in vivo MRS report
to examine the effect of a sedative hypnotic on
brain high energy phosphorous metabolism. Be-
cause brain MRS studies require that a patient
lie recumbent in the magnet for 2–4 hr, such
studies have been difficult to perform on elderly
patients, medically ill patients, psychiatric pa-
tients, and subjects with mild claustrophobia.
The ability to premedicate subjects with diaze-
pam will facilitate study of these populations
without significantly affecting ^{31}P MRS mea-
sures.

There are several limitations to the ^{31}P MRS
measurements. MRS volume selection tech-

niques such as ISIS are subject to a "resonance
offset" phenomenon that results in a slight shift
in the actual VOI for each metabolite. Second,
the tissue within the VOI was assumed to be
homogeneous. Third, the T_1s used to calculate
concentrations were derived from normal sub-
jects for large brain volumes that were less ho-
mogeneous than the VOIs of the current study.
Lastly, a uniform tissue water content was as-
sumed for all subjects. If the water content var-
ied among subjects, that could have affected the
concentrations; however, this could not have
accounted for our results as all metabolites would
have been affected equally. The limitations of
our methodology and the use of these simpli-
fying assumptions argues for caution in the
interpretation of our results.

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