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In vivo proton magnetic resonance spectroscopic examination of benzodiazepine action in humans

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ABSTRACT

In an examination of the effect of benzodiazepines on brain chemistry, 44 healthy controls underwent a short echo-time proton magnetic resonance spectroscopy (^{1}H MRS) session after induced sedation with intravenous midazolam (0.03 mg/kg) plus fentanyl (2 μg/kg). The regions of interest were the anterior cingulate cortex, right basal ganglia, right frontal lobe, and right hippocampus. Twenty-five of these subjects underwent the second ^{1}H MRS session while awake. The measured ^{1}H MRS metabolites included *N*-acetyl-aspartate, creatine-containing compounds (PCr+Cr), choline-containing compounds, *myo*-inositol, and glutamate plus glutamine, which were quantified both as absolute values and metabolite/PCr+Cr ratios. The results were analyzed using independent group *t* tests and repeated measures analysis of variance (ANOVA, with alpha values set at 0.025 to minimize the risk of false-positive findings arising from multiple comparisons. No significant difference between subjects under midazolam plus fentanyl induced sedation and awake could be detected with unpaired analyses. Paired comparisons by ANOVA with repeated measures found that neither drug (midazolam plus fentanyl) nor the drug by time (interval between two scan times) interaction had a significant effect on the quantified metabolites. These findings encourage utilization of benzodiazepine-induced brief sedation during in vivo ^{1}H MRS experiments of the brain, and may help with elucidation of state-dependent neurochemical alterations during the course of bipolar and schizoaffective disorders.

1. Introduction

Pathophysiological mechanisms underlying expression of psychiatric disorders are yet to be elucidated. Proton magnetic resonance spectroscopy (^{1}H MRS) allows assessment of neurochemical metabolites in vivo such as (i) *N*-acetylaspartate (NAA), which is a widely accepted marker for neuronal density, viability and function that is involved in myelin lipid turnover and mitochondrial energy production; (ii) phosphorylcholine plus glycerophospocholine (GPC+PC), which primarily reflects cell membrane phospholipids; (iii) phosphocreatine plus creatine (PCr+Cr), which represents cell energy metabolism; (iv) *myo*-inositol (mI), which participates in phospholipid metabolism and signal transduction; and (v) glutamate plus glutamine (Glu+Gln), which provides a window into the integrity of the glutamatergic synapse and neuronal–glial coupling (Auer et al., 2000; Yildiz-Yesiloglu and Ankerst, 2006; Moffett et al., 2007; Fountoulakis et al., 2008; Öngür et al., 2008; Scherk et al., 2009). Thus, along with 31P and 13C studies, hold considerable promise to illuminate brain mechanisms involved in severe psychiatric conditions such as bipolar and schizoaffective disorders (Kato et al., 1998; Glitz et al., 2002).

One of the major limitations of conducting in vivo MRS studies is the difficulty of scanning psychiatric patients when they are excited, agitated, or psychotic as in the case of bipolar or schizoaffective disorders in manic, mixed, or at times depressive states. During an MR scanning session, we need the patient subject to stay calm, quiet, and motionless for a reliable assessment of brain metabolites. However, patients in manic or mixed states or with psychotic symptoms are usually uncooperative. Considering presentation of bipolar or schizoaffective disorders in three, and unipolar depression in two different mood states, each with a potentially distinct neurochemical profile, better understanding of the pathophysiology would only be possible if we could obtain reliable and comparable information about

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brain chemistry during the mood states of mania and/or depression where accompanying psychosis and/or agitation is not uncommon.

Moderate sedation often induced by benzodiazepines is a level of anesthesia in which uncooperative patients are able to tolerate procedures requiring them not to move. Benzodiazepines interact with a specific binding site in the brain, which is an integral part of the gamma-aminobutyric acid$_A$ ($GABA_A$) receptor complex (Nutt and Malizia, 2001; Rudolph and Antkowiak, 2004). The ongoing level of neuronal activity is regulated by the balance between excitatory inputs (mostly glutamatergic) and inhibitory GABAergic activity. The $GABA_A$–benzodiazepine receptors, which are ligand-gated chloride channels, comprise five protein sub-units arranged around a central pore (Nutt and Malizia, 2001; Prommer, 2008). Benzodiazepine binding allosterically changes the receptor complex to increase the efficiency of GABA, so enabling the GABAergic circuits to produce a greater inhibitory effect. Compared with barbiturates, chloral hydrate, and ethanol, which can directly open the chloride channel, benzodiazepines are safer since the vital brain circuits cannot be inhibited over and above the level that would be achieved by the natural GABAergic effects (Nutt and Malizia, 2001).

Very few studies to date examined whether acute sedation with benzodiazepines could cause significant changes in the in vivo brain MR spectra. Brambilla et al. (2002) using ^{1}H MRS, and Deicken et al. (1992) using ^{31}P MRS have shown that benzodiazepines when administered orally do not change the human brain chemistry. We performed this study to examine whether parenteral administration of benzodiazepines does affect the human brain metabolite levels as measured by ^{1}H MRS. We examined the anterior cingulate cortex (ACC), right basal ganglia, right frontal lobe, and right hippocampus because earlier ^{1}H MRS studies of bipolar and schizoaffective disorders have reported changes suggestive of neuronal dysfunction in these brain regions (Strakowski et al., 1994; Soares and Mann, 1997; Yildiz-Yesiloglu and Ankerst, 2006; Moffett et al., 2007; Öngür et al., 2008; Scherk et al., 2009). The underlying *a priori* hypothesis was that the healthy subjects with and without benzodiazepine-induced transient sedation would have similar brain metabolite concentrations.

2. Methods

2.1. Subjects

Forty-four healthy human subjects who had no DSM-IV axis I disorder, as determined by the SCID-IV non-patient version (SCID-NP) were enrolled and underwent a ^{1}H MRS session under midazolam (0.03 mg/kg) plus fentanyl (2 μg/kg) intravenous (iv) administration. Of these 25 subjects, 44 underwent a second ^{1}H MRS session awake without administration of any drugs within the same study year (time interval between the two scans, mean ± standard deviation [SD]: 121.9 ± 86.7 days). They did not have any current medical problems or history of psychiatric disorders among their first-degree relatives. The study was approved by the Dokuz Eylül University, Institutional Ethics Committee. Written informed consent was obtained from all subjects.

2.2. Induction of sedation

The subjects who were scheduled for ^{1}H MRS under 'moderate sedation' fasted 6 h prior to the examination. For all subjects a venous route was opened with a 20-gauge iv cannula and a 0.9% normal saline infusion was initiated at the time of the scheduled MR scan. A study anesthesiologist who stayed with the subject throughout the procedure, administered midazolam 0.03 mg/kg iv, which was followed by fentanyl 2 μg/kg iv at a 1-min interval, just before the initiation of the MR session. Sedation was followed by the Ramsey Sedation Score and 0.015 mg/kg additional midazolam was administered until a Ramsey Sedation Score of 3 was reached (Ramsey et al., 1974). During the ^{1}H MRS sessions 6 L/min oxygen was applied via a face mask. Heart rate, respiratory rate, and Ramsey Sedation Score were monitored. At the end of the MRS session subjects with a Ramsey Sedation Score of 2 were transferred to the recovery room and post-anesthesia discharge scores were monitored at 10-min intervals. Subjects with a post-anesthesia score of 9 or higher were discharged. The mean discharge time was 1 h (Alderete Score) (Blanshard and Chung, 1999).

2.3. ^{1}H MRS procedures

In vivo ^{1}H MRS was conducted on a Philips System, at field strength of 1.5 T. The subjects were provided with earplugs to reduce noise disturbances, and the subject's head was positioned comfortably in the quadrature head radiofrequency coil with foam cushioning for motion stability. A set of sagittal, transverse and coronal T2 weighted images was obtained to verify subject position, image quality, and voxel positioning. To ensure consistent voxel placement, all scanner operators (who were certified MR technologists) were trained for this study and followed a standard prescription based on anatomic boundaries and reference atlas images for each region of interest. Anatomic (T2 weighted) images showing voxel placement were reviewed for consistency and accuracy for each region.

The single voxel short TE MRS data were collected with a PRESS sequence (TE = 31 ms, TR = 2000 ms, spectral bandwidth 1 kHz, 1024 complex data points, 128 acquisitions). Single voxel spectra were obtained from four different brain regions with voxel dimensions of 20 × 30 × 25 mm (15 cm^3) for the ACC; 15 × 30 × 20 mm (9 cm^3) for the right basal ganglia; 20 × 20 × 20 mm (8 cm^3) for the right frontal lobe; and 30 × 20 × 15 mm (9 cm^3) for the right hippocampus (Fig. 1). We used the commercial spectral-fitting package LC Model (version 6.1-4E) to measure metabolite peak integrals (Provencher, 1993). Unsuppressed water reference spectra were acquired for all acquisitions and used for both eddy-current correction and water-scaling to estimate absolute metabolite concentrations. Metabolite ratios, which are somewhat less sensitive to partial volume and relaxation effects, were also calculated for reference with the existing literature. T1 and T2 relaxation times were not measured. A long TR (2000 ms) and a short TE (31 ms) will significantly attenuate T1 and T2 relaxation effects, respectively. Only the ^{1}H MRS metabolites with reasonable precision for quantification (Stanley et al., 1995) were reported in the results (i.e., NAA, GPC+PC, mI, Glu+Gln PCr+Cr), and were expressed as absolute values, as well as metabolite/PCr+Cr ratios.

2.4. Data analysis

A two-pass quality assessment was made for all spectra. First, a global qualitative assessment of the spectral fit and baseline for each spectrum was made. Of the 276 potential in vivo proton brain spectra (four brain regions for 44 healthy subjects with 25 going through a 2nd scan), 241 were judged to be of adequate quality to undergo quantitative analysis. Following spectral fitting with the LC (linear combinations) Model, individual metabolite results were accepted/rejected based on specific "goodness of fit" criteria discussed below. We set a CRLB (Cramer-Rao lower bounds) threshold of less than 20% for NAA, GPC+PC, and PCr+Cr but allowed a larger uncertainty for Glu+Gln and mI: CRLB<40% due to the greater difficulty of fitting these metabolites.

The LC Model uses *a priori* knowledge (optimized linear combinations of single metabolite basis set spectra) for fitting *in vivo* spectral peaks which correspond to these basis spectra, and allows resolution of some cases of overlapping spectral peaks which can be accurately separated due to curve fitting and linear-combination constraints derived from fitting other satellite peaks (at different ppm) for each metabolite. In these cases the data would be reported and included in