Published in final edited form as:

Biol Psychiatry. 2009 February 1; 65(3): 273–275. doi:10.1016/j.biopsych.2008.06.023.

Prefrontal Cortical GABA Levels in Panic Disorder Determined by Proton Magnetic Resonance Spectroscopy

Gregor Hasler, M.D.¹, Jan Willem van der Veen, Ph.D.², Marilla Geraci, R.N.³, Jun Shen, Ph.D.², Daniel Pine, M.D.⁴, and Wayne C. Drevets, M.D.³

¹ Department of Psychiatry, University Hospital, 8091 Zurich, Switzerland ² National Institute of Mental Health, Magnetic Resonance Spectroscopy Core, National Institutes of Health, Bethesda 20892, U.S.A ³ National Institute of Mental Health, Mood and Anxiety Disorders Program, Section on Neuroimaging in Mood and Anxiety Disorders, National Institutes of Health, Bethesda 20892, U.S.A ⁴ National Institute of Mental Health, Mood and Anxiety Disorders Program, Section on Development and Affective Neuroscience, National Institutes of Health, Bethesda 20892, U.S.A

Abstract

Background—Panic disorder (PD) is hypothesized to be associated with altered function of the major inhibitory neurotransmitter, gamma-amino butyric acid (GABA). Previous proton magnetic resonance spectroscopy (MRS) studies found lower GABA concentrations in the occipital cortex of subjects with PD relative to healthy controls. The current study is the first MRS study to compare GABA concentrations between unmedicated PD subjects and controls in the prefrontal cortex (PFC).

Methods—Unmedicated subjects with PD (n=17) and age- and sex-matched healthy controls (n=17) were scanned on a 3 Tesla scanner using a transmit-receive head coil that provided a sufficiently homogenous radiofrequency field to obtain spectroscopic measurements in the dorsomedial/dorsal anterolateral and ventromedial areas of the PFC.

Results—The prefrontal cortical GABA concentrations did not differ significantly between PD subjects and controls. There also was no statistically significant difference in Glx, choline or N-acetyl-aspartate concentrations.

Conclusions—The previously reported finding of reduced GABA concentrations in the occipital cortex of PD subjects does not appear to extend to the PFC.

Keywords

gamma-amino butyric acid; anxiety disorders; MRS; glutamate; depression

Corresponding Author: Gregor Hasler, MD, Department of Psychiatry, University Hospital, Culmannstrasse 8, 8091 Zurich, Switzerland, Phone: +41 44 255-5280, Fax: +41 44 255-4408, Email address: g.hasler@bluewin.ch.

Financial Disclosure

The authors reported no biomedical financial interests or potential conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Panic disorder (PD) is hypothesized to be associated with altered function of the major inhibitory neurotransmitter, gamma-aminobutyric acid (GABA)⁽¹⁾. Goddard et al⁽²⁾ initially applied MRS to assess brain GABA concentrations in the occipital lobe of PD patients, and found that GABA levels were reduced in 12 of 14 unmedicated PD subjects⁽²⁾. Another study found decreased GABA levels in the anterior cingulate cortex and basal ganglia of medicated PD subjects⁽³⁾. Based upon these results, we hypothesized that GABA levels would be reduced in the prefrontal cortex in unmedicated PD subjects versus controls.

In vivo proton MRS is a valid tool for studying the concentrations of abundant neurotransmitters in brain disorders^(4,5). Because of the relatively low sensitivity of the imaging method, MRS spectra were acquired from two large voxels with a long scan time (27min per voxel). Based on benzodiazepine receptor studies in PD⁽⁶⁾ one voxel was positioned in the dorsomedial/ anterolateral PFC (DM/DALPFC); based on neurophysiological imaging studies in PD⁽¹⁾ a second voxel was positioned in the ventromedial PFC (VMPFC).

Methods and Materials

Participants between 18 and 60 years met DSM-IV criteria for PD [n=17, 12 female, mean age=34.2±10.1 years (range 21–55)]. Healthy control subjects with no history of any psychiatric disorder and no major psychiatric condition in first-degree relatives were matched for age and gender to the PD subjects [n=17, 12 female; mean age=35.1±11.8 years (range 19–58)]. Diagnosis was established by the Structured Clinical Interview for DSM-IV and confirmed by an unstructured interview with a psychiatrist. Exclusion criteria included major medical illnesses, pregnancy, psychotropic drug exposure (including nicotine and excessive alcohol consumption) within 3 months, lifetime history of substance abuse and dependence, psychiatric disorders other than PD, other anxiety disorders and MDD, structural brain abnormalities on MRI, or general MRI exclusions. Subjects were asked to abstain from alcohol within the 24 hours prior to scanning. The severity of panic, anxiety and depressive symptoms was assessed using the Panic Disorder Severity Scale (PDSS), the Panic Symptom Scale (PSS), the Hamilton Anxiety Scale (HAS) and the 21-item Hamilton Depression Rating Scale (HDRS). Written informed consent was obtained as approved by the NIMH IRB.

The methods for obtaining the MRS measures were identical to those used in our previous GABA-MRS studies, as detailed elsewhere⁽⁷⁾. Subjects were scanned (between 9/2004–11/2005) in a single session on a 3 Tesla GE scanner using a GE transmit-receive head coil capable of providing a homogenous radiofrequency field and spectroscopic measurements from prefrontal cortical tissue. Proton MRS spectra were acquired from two voxels: the DM/DALPFC voxel (5×3×2cm) and the VMPFC voxel (3×3×2cm). For more details see footnote of table 1.

GABA was measured using an interleaved PRESS-based J editing method^(8,9). The concentrations of GABA, choline, N-acetyl aspartate (NAA), and co-edited Glx (i.e., glutamate and glutamine) were expressed in mmol/liter (mM) referenced to concentration of creatine that was found to be normal in the medial prefrontal cortex of PD subjects⁽¹⁰⁾. Creatine was set to 7.1 mmol/L, a value which constituted the average concentration from literature reports of creatine in gray and white matter^(11,12). This conventional creatine referencing method has been used previously^(13,14), and its application in the current study was supported by previously published data showing no difference in the *medial PFC* creatine levels between PD subjects and healthy controls⁽¹⁰⁾. Gray matter fraction was derived from segmentation based on a histogram of anatomical image intensities.

The hypothesis that GABA concentrations in the VMPFC and DM/DALPFC would be reduced in PD subjects versus controls was tested using paired two-tailed t-tests. Because we tested this hypothesis in two regions, the significance threshold was set at a Bonferroni-adjusted significance level of $p=0.025$.

Results

In the PD group the mean age at PD onset was 22 ± 8.9 years, mean duration of PD was 108 ± 120 months, and mean time medication free was 21 ± 31 months (range 3–96 months; 9 subjects were drug-naïve). Seven PD subjects had comorbid MDD, seven had comorbid agoraphobia and three had a comorbid phobic disorder. The mean PDSS score was 7.5 ± 3.0 , the mean PSS score was 18 ± 5.4 , the mean HAS score was 9.6 ± 6.2 , and the mean HDRS score was 9.6 ± 7.4 .

The concentrations of GABA, Glx, choline, and NAA did not differ significantly between PD subjects and controls in either the DM/DALPFC or the VMPFC (table 1). The gray matter fraction was reduced in PD subjects relative to controls in the DM/DALPFC (40.3% versus 43.0%, respectively, $p=0.022$), but did not differ significantly between groups in the VMPFC ($p=0.56$). When the MRS measures were covaried for gray matter fraction, sex and age the study results remained unchanged. No significant diagnosis-by-sex interaction was identified. There was no significant difference in the creatine/NAA ratio between PD subjects and controls in either voxel ($p>.1$). The GABA levels did not differ between the seven PD+MDD subjects and controls, although the small sample size provided low power.

Discussion

These data constitute the first comparison of GABA concentrations in the PFC between unmedicated PD subjects and controls. The total GABA levels did not differ significantly between PD subjects and controls in the VMPFC or the DM/DALPFC. The PD subjects studied were either medication naïve ($n=9$) or unmedicated for ≥ 3 months, and were matched subject-by-subject to the controls for age and gender(9).

Several methodological limitations merit comment. By requiring that subjects had not been exposed to psychotropic drugs for ≥ 3 months, the sample was biased toward cases with PD of moderate severity. In addition, the regional MRS data were normalized to local creatine concentrations using a conventional creatine referencing method(13,14), but creatine levels were not independently assessed. Massana et al.(10) previously reported that creatine levels were reduced in the right medial temporal lobe region in PD, although this study found *no difference* in creatine levels between PD patients and healthy controls *in the medial PFC*. Finally, the menstrual phase was not characterized in the females subjects. Epperson et al. (15,16) recently reported effects of menstrual cycle on MRS-GABA measures in the occipital cortex. However, we did not detect a difference in GABA levels in the VMPFC or DM/DALPFC between eight healthy women imaged in the follicular phase versus eight women in the luteal phase (Hasler and van der Veen, unpublished data).

With 17 participants per group, an effect size (Cohen's d) of .99 is needed to provide 80% power to detect a difference at $\alpha=.05$. Using paired t tests, this translates to a difference in GABA levels of 16% in the DM/DALPFC and 13% in the VMPFC given the variance measured in our study. In the DM/DALPFC, Cohen's d was 0.0. In the VMPFC voxel, Cohen's d was 0.23, but the direction of difference was in the direction opposite to that hypothesized. Consequently, the likelihood that our negative results were explained by Type-II appeared low.

Our results differed from those Ham et al.(3), who found reduced GABA levels in the anterior cingulate cortex in PD subjects versus controls. This region was encompassed within the

VMPFC and DM/DALPFC voxels we placed, although the addition of more rostral PFC regions in our ROI would have diluted the effect of GABA levels from the cingulate cortex. Notably, all of the PD subjects studied by Ham et al. were receiving a combination of antidepressants and anxiolytics prior to scanning, while the PD subjects in our study were unmedicated. Since several classes of anxiolytic and antidepressant drugs alter central GABAergic transmission(17,18). The reduction in GABA levels reported in this previous study thus may have reflected the confounding effects of medications.

Our study also did not extend the previously reported finding of reduced GABA levels in the occipital cortex(2) to the PFC. However, some differences between the Goddard et al. study (2) and our study exist which require investigation in future studies. Compared with our study, the subjects in Goddard et al. had more severe panic and depressive symptoms (mean PDSS and 25-item HAM-D scores in that study were 13 and 20, respectively). Since GABA levels appear reduced in the occipital and prefrontal cortices of patients with major depressive disorder(7,13,19), it is conceivable that the reduced GABA levels Goddard et al. observed in their PD sample may have reflected comorbid depression, although the GABA levels and depression ratings were not correlated significantly in Goddard et al. Finally, although treatment withdrawal effects may have influenced occipital cortex GABA levels in Goddard et al.(2) since they included PD subjects following a minimum of one-week medication washout, 9 of their 14 PD subjects were medication naïve, reducing the likelihood that such effects accounted for their findings.

Given alterations of the GABA-A receptor system in PD and the effects of rapid anxiolytic drugs on the GABAergic system, GABA-ergic function appears relevant to the pathophysiology of PD(6). The GABA concentration measured using MRS predominantly reflects intracellular, rather than intrasynaptic, GABA concentrations, as the majority of the GABA pool exists within GABAergic neurons(20). Thus while our methods did not address whether GABA neurotransmission in the PFC may be altered in PD, we found no abnormalities in the total intracellular GABA pool averaged over a broad PFC area.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This research was supported by the Intramural Research Program of the National Institutes of Mental Health.

References

1. Charney, DS.; Drevets, WC. The Neurobiological Basis of Anxiety Disorders. In: Davis, KL.; Charney, DS.; Coyle, JT.; Nemeroff, CB., editors. Neuropsychopharmacology: The Fifth Generation of Progress. Philadelphia, PA: Lippincott Williams & Wilkins; 2002. p. 901-930.
2. Goddard AW, Mason GF, Almai A, Rothman DL, Behar KL, Petroff OA, et al. Reductions in occipital cortex GABA levels in panic disorder detected with 1h-magnetic resonance spectroscopy. Arch Gen Psychiatry 2001;58:556–561. [PubMed: 11386984]
3. Ham BJ, Sung Y, Kim N, Kim SJ, Kim JE, Kim DJ, et al. Decreased GABA levels in anterior cingulate and basal ganglia in medicated subjects with panic disorder: a proton magnetic resonance spectroscopy (1H-MRS) study. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:403–411. [PubMed: 17141385]
4. Ende G, Braus DF, Walter S, Weber-Fahr W, Soher B, Maudsley AA, Henn FA. Effects of age, medication, and illness duration on the N-acetyl aspartate signal of the anterior cingulate region in schizophrenia. Schizophr Res 2000;41:389–395. [PubMed: 10728716]

5. Sijens PE, den Heijer T, Origgi D, Vermeer SE, Breteler MM, Hofman A, Oudkerk M. Brain changes with aging: MR spectroscopy at supraventricular plane shows differences between women and men. *Radiology* 2003;226:889–896. [PubMed: 12601215]
6. Hasler G, Nugent AC, Carlson PJ, Carson RE, Geraci M, Drevets WC. Altered cerebral GABAA-benzodiazepine receptor binding in panic disorder determined by [11C]flumazenil PET. *Arch Gen Psychiatry*. 2008in press
7. Hasler G, van der Veen JW, Tumonis T, Meyers N, Shen J, Drevets WC. Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* 2007;64:193–200. [PubMed: 17283286]
8. Sailasuta N, LeRoux P, Hurd R, Wang P, Sachs N, Ketter T. Detection of cerebral gamma-aminobutyric acid (GABA) in bipolar disorder patients and healthy volunteers at 3T. *Proc Intl Soc Mag Reson Med* 2001:9.
9. Hasler G, Neumeister A, van der Veen JW, Tumonis T, Bain EE, Shen J, et al. Normal Prefrontal Gamma-Aminobutyric Acid Levels in Remitted Depressed Subjects Determined by Proton Magnetic Resonance Spectroscopy. *Biol Psychiatry* 2005;58:969–973. [PubMed: 16043137]
10. Massana G, Gasto C, Junque C, Mercader JM, Gomez B, Massana J, et al. Reduced levels of creatine in the right medial temporal lobe region of panic disorder patients detected with (1)H magnetic resonance spectroscopy. *Neuroimage* 2002;16:836–842. [PubMed: 12169267]
11. Hetherington HP, Mason GF, Pan JW, Ponder SL, Vaughan JT, Twieg DB, Pohost GM. Evaluation of cerebral gray and white matter metabolite differences by spectroscopic imaging at 4.1T. *Magn Reson Med* 1994;32:565–571. [PubMed: 7808257]
12. Kreis R, Ernst T, Ross BD. Absolute quantification of water and metabolites in the human brain: II. Metabolite concentrations. *J Magn Reson B* 1993;102:9–19.
13. Sanacora G, Mason GF, Rothman DL, Behar KL, Hyder F, Petroff OA, et al. Reduced cortical gamma-aminobutyric acid levels in depressed patients determined by proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* 1999;56:1043–1047. [PubMed: 10565505]
14. Shen J, Rothman DL, Brown P. In vivo GABA editing using a novel doubly selective multiple quantum filter. *Magn Reson Med* 2002;47:447–454. [PubMed: 11870830]
15. Epperson CN, O'Malley S, Czarkowski KA, Gueorguieva R, Jatlow P, Sanacora G, et al. Sex, GABA, and nicotine: the impact of smoking on cortical GABA levels across the menstrual cycle as measured with proton magnetic resonance spectroscopy. *Biol Psychiatry* 2005;57:44–48. [PubMed: 15607299]
16. Epperson CN, Haga K, Mason GF, Sellers E, Gueorguieva R, Zhang W, et al. Cortical gamma-aminobutyric acid levels across the menstrual cycle in healthy women and those with premenstrual dysphoric disorder: a proton magnetic resonance spectroscopy study. *Arch Gen Psychiatry* 2002;59:851–858. [PubMed: 12215085]
17. Goddard AW, Mason GF, Appel M, Rothman DL, Gueorguieva R, Behar KL, Krystal JH. Impaired GABA neuronal response to acute benzodiazepine administration in panic disorder. *Am J Psychiatry* 2004;161:2186–2193. [PubMed: 15569888]
18. Bhagwagar Z, Wylezinska M, Taylor M, Jezzard P, Matthews PM, Cowen PJ. Increased Brain GABA Concentrations Following Acute Administration of a Selective Serotonin Reuptake Inhibitor. *Am J Psychiatry* 2004;161:368–370. [PubMed: 14754790]
19. Sanacora G, Gueorguieva R, Epperson CN, Wu YT, Appel M, Rothman DL, et al. Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. *Arch Gen Psychiatry* 2004;61:705–713. [PubMed: 15237082]
20. Petroff OA. GABA and glutamate in the human brain. *Neuroscientist* 2002;8:562–573. [PubMed: 12467378]

Table 1
Metabolite Concentrations (mmol/liter) in Prefrontal Brain Regions^a

Voxel	Subjects	GABA	Co-edited Glx ^c	Choline	NAA
DM/DALPFC ^b	PD subjects (N=17)	0.98 (0.18)	2.57 (0.48)	1.59 (0.22)	10.32 (0.54)
	Healthy controls (N=17)	0.98 (0.25)	2.59 (0.48)	1.61 (0.32)	9.92 (0.99)
	Statistics (df=16)	t=0.07 p=0.95	t=0.13 p=0.90	t=0.31 p=0.76	t=-1.64 p=0.12
VMPFC ^b	PD subjects (N=17)	0.98 (0.16)	2.65 (0.37)	1.49 (0.25)	9.59 (0.90)
	Healthy controls (N=17)	0.94 (0.18)	2.78 (0.43)	1.62 (0.20)	9.79 (0.64)
	Statistics (df=16)	t=0.94 p=0.36	t=0.96 p=0.35	t=1.89 p=0.08	t=0.69 p=0.50

^aThe creatine concentration was set to 7.1 mmol/L. Metabolite concentrations are indicated as mean (standard deviation).

^bThe DM/DALPFC voxel extended 5×3×2cm and was positioned with the posterior border abutting the anterior pole of the caudate nucleus, and the ventral border abutting the dorsal border of the putamen. The VMPFC voxel extended 3×3×2cm and was positioned with the posterior border abutting the rostrum of the corpus callosum, and was centered in horizontal planes on the midline and in sagittal planes on the bicommissural line. An image displaying the voxel positions within the brain appears in (8).

^cThe co-edited Glx is proportional to the total Glx (see methods in (7)). The variation in Glx editing efficiency was estimated to be less than 22.9% based on the magnitude of the residual NAA signal in the edited subspectra.

Abbreviations: DM/DALPFC, dorsomedial/dorsal anterolateral prefrontal cortex region-of-interest; GABA, gamma-aminobutyric acid; Glx, glutamate/glutamine; NAA, N-acetyl aspartate; MDD, major depressive disorder; VMPFC, ventromedial prefrontal cortex region-of-interest