Neuroactive Steroid Levels in Patients With Generalized Anxiety Disorder

Trent Semeniuk, M.D.
Gian S. Jhangri, M.Sc.
Jean-Michel Le Mellédo, M.D.

Serum levels of allopregnanolone, pregnenolone sulfate, and dehydroepiandrosterone sulfate were measured in 8 male patients with generalized anxiety disorder (GAD) and 8 healthy control subjects. Results suggest that patients with GAD have significantly lower levels of pregnenolone sulfate than control subjects.

The Journal of Neuropsychiatry and Clinical Neurosciences 2001; 13:396–398)

Generalized anxiety disorder (GAD) is a common affliction, with a lifetime prevalence rate of 5.7% in males and 9.2% in females.[1] Several neurotransmitter systems have been suspected to play a role in the pathophysiology of GAD. Various lines of research suggest dysregulation of the gamma-aminobutyric acid ($GABA_A$) / benzodiazepine (BZ) receptor complex. These findings include decreased numbers and sensitivities of BZ receptors in patients with GAD and successful pharmacologic treatment of GAD with agents that target the $GABA_A$ / BZ receptor (e.g., benzodiazepines).[2]

A number of compounds termed neuroactive steroids (NAS) that have intrinsic activity on the $GABA_A$ / BZ receptor have been characterized.[3] These compounds include the progesterone metabolite 5α-pregnan-3α-ol-20-one (allopregnanolone; ALLO), pregnenolone sulfate (PREGS), and dehydroepiandrosterone sulfate (DHEAS). In animal models, positive allosteric $GABA_A$ receptor modulators such as ALLO display anxiolytic activity,[4,5] whereas negative $GABA_A$ allosteric modulators such as DHEAS may exhibit anxiogenic activity.[6]

The aim of this study was to compare serum levels of ALLO, PREGS, and DHEAS between healthy control subjects and medication-free patients with GAD and no other concurrent psychiatric diagnosis. The hypothesis tested was that patients with GAD have lower levels of NAS with $GABA_A$ /BZ receptor agonist activity and /or higher levels of NAS with antagonist activity.

METHODS

Subjects

The research study was approved by the University of Alberta Hospital Ethics Committee, and all participants gave both verbal and written informed consent prior to participating in the study. Eight male patients with GAD and eight healthy male control subjects (mean age 38.13 ± 10.68 years and 34.25 ± 8.48 years, respectively; $t = 0.804$, df = 14, $P = 0.44$ by independent t-test; means reported with standard deviations) were recruited from the community via advertisements. All subjects were screened with the Mini-International Neuropsychiatric Interview[7] and all patients met the DSM-IV diagnostic criteria for GAD. The mean Hamilton Anxiety Scale score was 24.25 ± 7.25 in patients and 2.88 ± 3.31 in control subjects. Subjects were free of any psychotropic medications for a minimum of 2 weeks prior to the study, and no subjects were on fluoxetine prior to the study. Exclusion criteria for both patients and control subjects were any significant medical illness, substance abuse, or the use of any hormonal medications. Control subjects were also excluded if they had a past history of any psychiatric disorders. Additional exclusion criteria for patients were any current psychiatric disorder other than GAD.

Neuroactive Steroid Analyses

Dr. R. Gupta (SciMed Labs, Edmonton, AB) performed all NAS analyses. For the analysis of ALLO and PREGS, 2 to 3 ml of plasma was extracted three times with ethyl acetate (1:1 ratio) and the organic phase was then lyophilized. The residue was dissolved in 1 ml of methanol, and 100 µl of the prepared sample was analyzed by high performance liquid chromatography (Hewlett-Packard HPLC 1100), using a C18 Symmetry column (3.9×150 mm, 5 µm; Waters Corp., Milford, MA) attached to the guard column (C18, ODS, Octadecyl, 4 mm × 3 mm; Phenomenex, Torrance, CA). The mobile

Received June 14, 2000; revised September 13, 2000; accepted September 20, 2000. From the Department of Psychiatry, 1E1.01 Mackenzie Centre, University of Alberta Hospital, Edmonton, Alberta, T6G 2B7, Canada. Address correspondence to Dr. Semeniuk. E-Mail: tsemeniu@ualberta.ca.

phase varied from 20% HPLC grade acetonitrile (ACN) to 80% ACN delivered at a rate of 1.0 ml/min. The UV monitor was set at 200 nm. Authentic samples of the NASs (Sigma, Oakville, ON) were used for preparation of calibration curves. Control samples were run along with the test samples at regular intervals for consistent reliability of the data throughout the assay. Concentrations of unknown samples were determined automatically by using Microsoft Excel. The retention time for ALLO was 12.25 minutes at 75% ACN, and that for PREGS was 6.6 minutes at 60% ACN. The coefficient of variation was less than 10% for both ALLO and PREGS.

DHEAS levels were quantitated by an enzyme immunoassay. Five calibration points and two sets of control sera were used for analyzing the samples. Control subjects were positioned at regular intervals to maintain the consistency of the data throughout the assay. The samples were incubated with enzyme-labeled antigen (Diagnostic Systems Laboratories, Inc., Webster, TX) to compete for their antibody binding sites. The unbound materials were washed off, and DHEAS concentrations were measured at 450 nm by the hydrolysis of the enzyme-substrate chromogen. The coefficient of variation was 2% to 4% and the r^2 value was 0.99.

Data Analysis

The NAS measures between patients and control subjects were compared by two-tailed independent *t*-tests. *P*-values less than 0.05 were considered significant.

RESULTS

The average level of ALLO in patients with GAD (3.78 ± 0.92 ng/ml) was lower compared with control subjects (9.91 ± 9.27 ng/ml) but did not reach statistical significance ($t = 1.86$, df = 7,14, $P = 0.10$). Levels of PREGS were significantly lower in patients than in control subjects (4.26 ± 4.49 ng/ml vs. 12.60 ± 7.07 ng/ml; $t = 2.82$, df = 14, $P = 0.014$). There was no significant difference in the levels of DHEAS between patients and control subjects ($2{,}259 \pm 1{,}703$ ng/ml vs. $3{,}396 \pm 1{,}708$ ng/ml; $t = 1.33$, df = 14, $P = 0.20$).

DISCUSSION

The finding of statistically significant lower levels of PREGS in patients with GAD is consistent with in vitro binding experiments, which found PREGS to act as an agonist on the $GABA_A$ receptor at low concentrations,[8,9] and with in vivo experiments, which found that intraperitoneal administration of low-dose PREGS to mice can lead to a reduction of anxiety.[10] However, in those experiments[9,10] it was also noted that PREGS could act as a $GABA_A$ receptor antagonist and functional anxiogenic agent at higher doses.

Although the lower level of ALLO in patients was only statistically suggestive, the direction of the results would indicate a decrease in an endogenous anxiolytic agent and lower agonist activity at the level of the $GABA_A$/BZ receptor in patients with GAD.

The clinical relevance of our results is supported by findings of abnormal cerebral spinal fluid (CSF) and serum levels of NAS in patients with major depressive disorder.[11,12] It has been observed that selective serotonin reuptake inhibitors (SSRIs) normalized these abnormal NAS levels, possibly by altering the kinetics of enzymes involved in NAS metabolism.[13] On the basis of preliminary reports on the efficacy of SSRIs in GAD,[14] we suggest, and are currently testing the hypothesis, that treatment with SSRIs normalizes NAS levels in GAD patients.

The relevance of plasma levels of neurochemicals to central nervous system activity is always difficult to determine. It has been shown, however, that PREGS crosses the blood–brain barrier,[15] a finding that supports the clinical relevance of plasma PREGS alterations. A second limitation on the interpretation of the results is the small sample size in this study, which is explained by the relative rarity of unmedicated male patients with GAD who do not present with a comorbid Axis I psychiatric disorder. However, in spite of the limitations, our findings are suggestive of different levels of certain neuroactive steroids in patients with GAD. These findings will require confirmation in a larger study.

The authors thank Annette Glowacki, Glen Baker, Janisse Khudabux, Karen McManus, Wendy Chrapko, Nathalie Lara, and Darin Bailey. Funding for this study was provided by the Alberta Heritage Foundation for Medical Research (file 199700226 and salary award to J-M.L.) and the Medical Research Council of Canada (grant MA-15008 to J-M.L.). The results of this study were presented at the 23rd annual meeting of the Canadian College of Neuropsychopharmacology, Marrakech, Morocco, April 10–13, 2000, and the 22nd Congress of the Collegium Internationale Neuro-Psychopharmacologicum, Brussels, Belgium, July 9–13, 2000.

References

1. Gater R, Tansella M, Korten A, et al: Sex differences in the prevalence and detection of depressive and anxiety disorders in general health care settings. Arch Gen Psychiatry 1998; 55:405–413
2. Connor KM, Davidson JRT: Generalized anxiety disorder: neurobiological and pharmacotherapeutic perspectives. Biol Psychiatry 1998; 44:1286–1294

3. Rupprecht R, Holsboer F: Neuroactive steroids: mechanisms of action and neuropsychopharmacological perspectives. Trends Neurosci 1999; 22:410–416
4. Bitran D, Hilvers RJ, Kellogg CK: Anxiolytic effects of 3α-hydroxy-5 alpha[beta]-pregnan-20-one: endogenous metabolites of progesterone that are active at the $GABA_A$ receptor. Brain Res 1991; 561:157–161
5. Bitran D, Purdy RH, Kellogg CK: Anxiolytic effect of progesterone is associated with increases in cortical allopregnanolone and $GABA_A$ receptor function. Pharmacol Biochem Behav 1993; 45:423–428
6. Reddy DS, Kulkarni SK: Differential anxiolytic effects of neurosteroids in the mirrored chamber behavior test in mice. Brain Res 1997; 752:61–71
7. Sheehan DV, Lecrubier Y, Sheehan KH, et al: The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 1998; 59(suppl)20:22–33
8. Gee KW, Joy DS, Belelli D: Complex interactions between pregnenolone sulfate and the *t*-butylbicyclophosphorothionate-labeled chloride ionophore in rat brain. Brain Res 1989; 482:169–173
9. Majewska MD, Schwartz RD: Pregnenolone-sulfate: an endogenous antagonist of the gamma-aminobutyric acid receptor complex in brain? Brain Res 1987; 404:355–360
10. Melchior CL, Ritzmann RF: Pregnenolone and pregnenolone sulfate, alone and with ethanol, in mice on the plus-maze. Pharmacol Biochem Behav 1994; 48:893–897
11. Ströhle A, Romeo E, Hermann B, et al: Concentrations of 3α-reduced neuroactive steroids and their precursors in plasma of patients with major depression and after clinical recovery. Biol Psychiatry 1999; 45:274–277
12. Uzunova V, Sheline Y, Davis JM, et al: Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. Proc Natl Acad Sci USA 1998; 95:3239–3244
13. Griffin LD, Mellon SH: Selective serotonin reuptake inhibitors directly alter activity of neurosteroidogenic enzymes. Proc Natl Acad Sci USA 1999; 96:13512–13517
14. Rocca P, Fonzo V, Scotta M, et al: Paroxetine efficacy in the treatment of generalized anxiety disorder. Acta Psychiatr Scand 1997; 95:444–450
15. Wang M, Wahlström G, Bäckström T: The regional brain distribution of the neurosteroids pregnenolone and pregnenolone sulfate following intravenous infusion. J Steroid Biochem Mol Biol 1997; 62:199–306