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Decreased Cerebrospinal Fluid Allopregnanolone Levels in Women with Posttraumatic Stress Disorder

Ann M. Rasmusson, Graziano Pinna, Prashni Paliwal, David Weisman, Christopher Gottschalk, Dennis Charney, John Krystal, and Alessandro Guidotti

Background: Alterations in the γ-amino-butyric acid (GABA) neurotransmitter system have been identified in some populations with posttraumatic stress disorder (PTSD).

Methods: To further investigate factors of relevance to GABAergic neurotransmission in PTSD, we measured cerebrospinal fluid (CSF) levels of allopregnanolone and pregnanolone combined (ALLO: congeners that potently and positively modulate effects of GABA at the $GABA_A$ receptor), 5α-dihydroprogesterone (5α-DHP: the immediate precursor for allopregnanolone), dehydroepiandrosterone (DHEA: a negative modulator of $GABA_A$ receptor function), and progesterone with gas chromatography, mass spectrometry in premenopausal women with (n = 9) and without (n = 10) PTSD. Subjects were free of psychotropic medications, alcohol, and illicit drugs; all were in the follicular phase of the menstrual cycle except three healthy and four PTSD subjects receiving oral contraceptives.

Results: There were no group differences in progesterone, 5α-DHP, or DHEA levels. The PTSD group ALLO levels were < 39% of healthy group levels. The ALLO/DHEA ratio correlated negatively with PTSD re-experiencing symptoms (n = −.82, p < 008; trend) and with Profile of Mood State depression/dejection scores (n = −0.70, p < 0008).

Conclusion: Low CSF ALLO levels in premenopausal women with PTSD might contribute to an imbalance in inhibitory versus excitatory neurotransmission, resulting in increased PTSD re-experiencing and depressive symptoms.

Progress has been made in treating posttraumatic stress disorder (PTSD) (Aulbucher and Liberzon 2002; Friedman 2002; Resick et al 2002), but the need for improved methods of PTSD prevention and treatment remains and likely will be met only as we expand our understanding of the pathophysiology of PTSD. Research regarding the neurobiology of PTSD thus far has revealed the complexity of the disorder, with reported PTSD-associated alterations in the monoaminergic, corticotropin-releasing factor (CRF), and neuropeptide Y (NPY) transmitter systems (Nutt and Malizia 2004; Southwick et al 2005) as well as in hypothalamic-pituitary-adrenal (HPA) axis (Rasmusson et al 2003; Yehuda et al 2002), thyroid (Karlovic et al 2004; Wang and Mason 1999), and immune system function (Baker et al 2001).

A variety of alterations in the γ-amino-butyric acid (GABA) neurotransmitter system also have been identified in PTSD. Decreased frontal lobe benzodiazepine receptor binding was found in male Viet Nam but not Gulf War veterans with chronic PTSD (Bremner et al 2000a; Fujita et al 2004). Decreased plasma GABA levels were associated with PTSD development in motor vehicle accident victims (Vaiva et al 2004). A polymorphism in the gene for the $GABA_A$ receptor $\beta3$ subunit was associated with higher levels of somatic symptoms, depression, anxiety, and

insomnia in PTSD (Feusner et al 2001). In addition, combat veterans with PTSD (Spivak et al 2000) and refugees from Kosovo who developed PTSD with greater sleep disturbance (Sondergaard et al 2002) showed increased plasma levels of dehydroepiandrosterone (DHEA) and/or its sulfated metabolite (DHEAS), androgenic steroids with negative modulatory effects at brain $GABA_A$ receptors. Premenopausal women with chronic PTSD also have shown greater DHEA release after maximal adrenal stimulation by adrenocorticotropic hormone ($ACTH_{1-24}$) administration (Rasmusson et al 2004).

In the current study, we further investigated the GABAergic neurotransmitter system in PTSD by measuring, in the cerebrospinal fluid (CSF) of premenopausal women with chronic PTSD, selected neuroactive steroids that influence the expression or function of brain $GABA_A$ receptors. Two of these steroids, the 3-α-reduced biosynthetic derivatives of progesterone, 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) and its stereoisomer, 3α-hydroxy-5β-pregnan-20-one (pregnanolone) (collectively termed ALLO for this paper), are the most potent and selective positive endogenous modulators of the action of GABA at brain $GABA_A$ receptors (Lambert et al 2003; Puia et al 1990). In addition, these steroids exert anxiolytic, sedative, and anesthetic effects at nanomolar concentrations (Paul and Purdy 1992), suggesting that they would be logical candidates for a role in the pathophysiology of PTSD.

Methodological challenges have limited previous studies of ALLO in PTSD and other clinical disorders. Radioimmunoassays lack the specificity and sensitivity needed to accurately measure ALLO in the CSF or in the plasma of men, follicular phase premenopausal women, postmenopausal women, and children. Therefore, we used gas-chromatography, mass-spectrometry (GC-MS), a method that allows structural identification and quantification of the neuroactive steroids of interest at femtomolar levels (Uzunova et al 1998). We measured (Figure 1): 1) ALLO; 2) 5α-dihydroprogesterone (5α-DHP), the immediate precursor for ALLO that acts with nanomolar potency at progesterone receptors that in turn interact with DNA response elements involved in modifying $GABA_A$ receptor expression (Canonaco et al 1989; Rupprecht 2003; Rupprecht et al 1993; Schumacher et al

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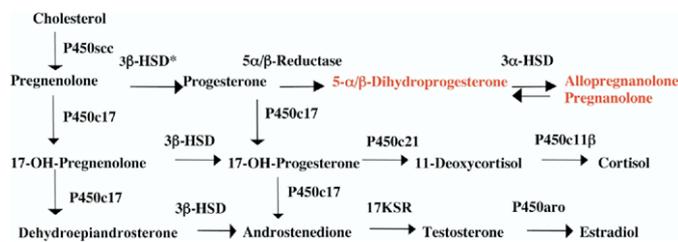Received August 10, 2005; revised March 3, 2006; accepted March 7, 2006.

Figure 1. Biosynthetic pathways for brain and/or peripheral neuroactive steroids. HSD, hydroxysteroid dehydrogenase; KSR; ketosteroid reductase; aro, aromatase.

1989); 3) progesterone (PROG), the immediate precursor for 5α-DHP; and 4) dehydroepiandrosterone (DHEA), a peripherally derived androgen (Compagnone and Mellon 2000) with negative modulatory effects at brain GABA$_A$ receptors at micromolar concentrations (Park-Chung et al 1999). Dehydroepiandrosterone also has positive modulatory effects at N-methyl-D-aspartate (NMDA) receptors, activity at sigma receptors, and antiglucocorticoid as well as neuroprotective properties (Baulieu and Robel 1998).

On the basis of the previous studies' finding of high DHEA or DHEAS levels or reactivity in PTSD and the opposing modulatory effects of DHEA(S) and ALLO at GABA$_A$ receptors, we hypothesized that CSF ALLO levels would be low and DHEA levels high in women with PTSD. Because the pharmacological properties of these neuroactive steroids might allow them to directly modulate psychiatric symptoms, we also investigated their relationship to clusters of related DSM-IV–defined PTSD symptoms identified by confirmatory factor analysis (Simms et al 2002) (Table 3). We hypothesized that CSF ALLO levels would correlate negatively with the re-experiencing and hyperarousal symptom clusters and that CSF DHEA levels would correlate negatively with the dysphoria symptom cluster (Table 3).

Methods and Materials

Subjects

Twenty-seven subjects were recruited for the study via community advertisements. The protocol was approved by the VA Connecticut Healthcare System Human Studies Subcommittee and the Yale Human Investigation Committee; written informed consent was obtained from each subject.

Subjects were screened for current or significant past medical illness and illicit drug use via medical history, physical examination, and laboratory tests. None had identifiable endocrine syndromes, and all reported regular menses on admission to the study.

Subjects were free from psychotropic medications for months to years or had never been treated with such. Subjects abstained from other medications, alcohol, and illicit drugs for at least 4 weeks before lumbar puncture except for four PTSD and three healthy, nontraumatized subjects who used oral contraceptives. One PTSD subject smoked approximately one-half pack of cigarettes per day.

The DSM-IV PTSD Criterion A1/A2 trauma exposure (American Psychiatric Association 1994) was assessed with general and early trauma inventories (ETI) (Bremner et al 2000b) (Table 1). To qualify as a Criterion A2 trauma, physical or sexual trauma ascertained by the ETI had to be scored as "very" or "extremely" distressing and disruptive in at least two of three functional domains (emotional, interpersonal, or school performance).

Healthy, nontraumatized subjects were evaluated psychiatrically with the non-patient edition of the Structured Clinical Interview for DSM-IV (SCID-NP) (First et al 1995a); subjects with a current Axis I diagnosis were excluded. The PTSD subjects were evaluated with the SCID-P (First et al 1995b); subjects with current or past psychotic disorders or bipolar disorder were excluded.

Menstrual Cycle Monitoring

Menstrual cycle phase was determined by use of a urine test kit that detects the mid-cycle luteinizing hormone surge (Clear Plan Easy, Whitehall Laboratories, Madison, New Jersey) and measurement of plasma progesterone levels 10–14 days later to confirm ovulation. Progesterone levels also were measured in the hospital clinical laboratory at the time of the lumbar puncture to confirm menstrual phase.

Lumbar Puncture

Subjects fasted except for water intake and abstained from smoking (in the case of the one PTSD subject who smoked) after midnight before the lumbar puncture. Subjects arrived at the Biostudies Unit at 7:30 AM, provided urine for a urine toxicology screen and β-HCG pregnancy testing, and then lay flat. Blood was drawn from an antecubital vein at −30 and −15 min before the lumbar puncture, which was performed between 8:30 and 9:30 AM. The CSF (25 cc) was collected in 1 cc aliquots into polypropylene tubes that were immediately placed on dry ice before storage in a −70° freezer. Blood samples were spun immediately in a refrigerated centrifuge and plasma was stored at −70°C.

Symptom Ratings

The Profile of Mood States scale (POMS; Educational and Industrial Testing Service, San Diego, California) was rated for the past week by all subjects during the 30 min before lumbar puncture. The Clinician Administered PTSD Scale (CAPS-One Week Version; Blake et al 1993) was administered after the lumbar puncture so as not to influence CSF neuroactive steroid levels by overtly stressing the PTSD subjects with trauma reminders.

Neuroactive Steroid Measurements

The CSF neuroactive steroid levels were measured with GC-MS. The detection limit for ALLO and the other neurosteroids with this method was approximately 1.0 fmol/mL, and the standard curve was linear between 1 and 10^5 fmol/mL The intra- and inter-assay coefficients of variation for this method are low (Uzunov et al 1996). Neuroactive steroid measurements were made in CSF aliquot 18 and confirmed either in aliquot 18 or 19. For one subject, CSF was available only from aliquot 19. Average values from multiple measurements of ALLO (either 2 or 3) were used in the data analyses for 15 subjects; only one measurement was available for four subjects. The PROG, 5α-DHP, and DHEA measurements were made once for each subject.

The CSF samples (700 μL) were extracted in ethylacetate and lyophilized; the neurosteroids of interest then were purified and separated with high-performance liquid chromatography (HPLC). Tritiated neurosteroids (New England Nuclear, Boston, Massachusetts) were added to monitor retention time and steroid recovery through the HPLC procedure (Cheney et al 1995), while deuterated internal standards (Cambridge Isotope Laboratories, Andover, Massachusetts) were added to allow quantification of the compound of interest. The HPLC-eluted fractions containing each steroid of

Table 1. Demographic Characteristics, PTSD A1, A2 Trauma Exposure, and DSM-IV Psychiatric Diagnoses of Subjects

Demographic Variable	PTSD Subjects (n = 9)		Healthy Nontraumatized Subjects (n = 10)		Group Difference
Age	Mean: 33.6 ± 6.1 yrs		Mean: 28.7 ± 8.6 yrs		p < .04[a]
	Range: 25–43 yrs		Range: 19–43 yrs		
Weight	Mean: 173.3 ± 33.3 lbs		Mean: 169.7 ± 43.5 lbs		p > .96
Education	Mean: 14.0 ± 2.0 yrs		Mean: 15.5 ± 1.8 yrs		p > .16
Ethnicity	Caucasian: n = 6; 66.7%		Caucasian: n = 4; 40%,		
	African American: n = 2; 22.2%		African American: n = 2; 20%,		
	Hispanic: n = 1; 11.1%		Hispanic: n = 1; 10%,		
			Asian: n = 2; 20%,		
			Askenazi Jewish: n = 1; 10%		
Trauma Exposure	Age 1st Trauma: All pre-adolescent		None		Yes, by definition.
	All with multiple traumas (#2–13)				
	All suffered sexual assault at least once				
Psychiatric Diagnoses (No. subjects)		Current/Past		Current/Past	Yes, by definition.
	Major Depression	4/3	Bulimia[a]	0/1	
	Dysthymia	3/0			
	Panic Disorder	2/0			
	Simple Phobia	2/0			
	Social Phobia	1/0			
	Agoraphobia w/o panic	2/1			
	Generalized Anxiety	1/1			
	OCD (cleaning only)	1/0			
	Bulimia	0/1			
	Anorexia	0/1			
	Alcohol Dependence[a]	0/2			
	Marijuana Dependence[a]	0/2			

PTSD, posttraumatic stress disorder; OCD, obsessive compulsive disorder.

[a] > 6 years before study participation (PTSD); > 10 years before study participation (Healthy).

interest then were derivatized for GC-MS (Dong et al 2001; Uzunova et al 1998). Because deuterated DHEA is not available, deuterated allopregnanolone was added to the HPLC-eluted fraction containing DHEA before derivatization. Mass spectrometry analysis was conducted in the standard electron impact mode (EI) for progesterone, ALLO, and DHEA and in the negative ion chemical ionization mode (NICI) with methane as the reaction gas for 5α-DHP. To calculate the quantity of the neurosteroid of interest in each fraction, the area under the peak of the neurosteroid in the sample was divided by the area under the peak of the deuterated internal standard. For DHEA, the ratio of the area under the DHEA peak to be determined divided by the area under the derivatized ALLO peak was plotted against similar ratios generated in a standard curve prepared by combining known quantities of authentic DHEA (1–200 fmol) with 100 fmol of deuterated ALLO. Further methodological details may be obtained by contacting the authors.

As previously shown, only the GABA_A receptor active 3α5α or 3α5β stereoisomers of ALLO are measurable in CSF, whereas the 3β5α and 3β5β isomers that antagonize the effects of the 3α isomers at high concentrations are below the limits of detection (Uzunova et al 1998). The 3α5α and 3α5β stereoisomers of ALLO were measured together in the current study, because the content of each in CSF is almost identical (Uzunova et al 1998); they are equipotent in modulating GABA action at GABA_A receptors, and separating them is very time consuming owing to their similar retention times. In the current study, they were eluted with a retention time of approximately 14 min and were easily separated from the 3β5α and 3β5β stereoisomers that have a retention time of approximately 16 minutes.

Statistical Analyses

The following subjects were excluded from the data analyses: one nontraumatized subject diagnosed with Adjustment Disorder with Depressed Mood in the context of currently stressful family circumstances, one traumatized subject meeting criteria for past and current partial PTSD, a healthy subject from whom CSF could not be obtained during the lumbar puncture, two PTSD and three healthy subjects in the luteal phase of the menstrual cycle at the time of lumbar puncture, and a healthy subject who initially reported regular menses but who failed to ovulate over the next 3 months.

For the purposes of data analysis, subjects receiving oral contraceptives (three healthy and four PTSD subjects) were considered in combination with subjects in the natural follicular phase of the menstrual cycle, because plasma progesterone levels are similar in these states. Analyses were therefore conducted in a total of 9 PTSD and 10 healthy subjects.

Because possible relationships between age, weight, or CSF progesterone levels and CSF levels of the neuroactive steroids of interest have not been defined previously, we performed exploratory Spearman correlations to ascertain whether these factors should be included as covariates in analyses used to compare neuroactive steroid levels between groups.

Nonparametric Mann–Whitney tests were initially used to compare means of the neuroactive steroid levels between groups. Linear regression was used to adjust for age and CSF progesterone levels on the basis of the exploratory analyses. Biologically relevant ratios between CSF levels of selected neuroactive steroids also were computed and compared between the PTSD and healthy groups. The ratio of ALLO to DHEA was calculated as a measure of balance between inhibitory and excitatory influences in the central nervous system (CNS). Ratios between progesterone and 5α-DHP and between 5α-DHP and ALLO were calculated to index possible

Table 2. Group Differences in CSF Neuroactive Steroid Levels and Ratios

	Findings Relevant to the Pathway for Allopregnanolone Synthesis					
	PTSD (n = 9)		HEALTHY (n = 10)		Unadjusted Comparison	Adjusted[a] Comparison
Neuroactive Steroid Measure	Mean (fmol/mL)	SEM	Mean (fmol/mL)	SEM	Mann Whitney p	Linear Regression p
PROG	439.6	112.0	392.5	106.6	.84	
5-α-DHP	282.5	69.6	152.1	23.4	.27	
ALLO[b]	14.1	2.6	36.2	6.6	.013[b]	$p < .01$[b]
PROG/5-α-DHP	2.91	.94	2.82	.61	.780	
5-α-DHP/ALLO[b]	28.8	9.8	5.1	.92	.006[b]	$p < .04$[b]

	Findings Regarding the Balance Between Neuroactive Steroids that Positively and Negatively Modulate GABA$_A$ Receptor Function					
	PTSD (n = 9)		HEALTHY (n = 10)		Unadjusted Comparison	Adjusted[a] Comparison
Neuroactive Steroid Measure	Mean (fmol/mL)	SEM	Mean (fmol/mL)	SEM	Mann Whitney p	Linear Regression p
DHEA	220.6	34.1	212.3	46.9	.60	
ALLO/DHEA[b]	.08	.02	.22	.06	.008[b]	$p < .03$[b]

PROG, progesterone; DHP, dihydroprogesterone; ALLO, combined allopregnanolone and pregnanolone; DHEA, dehydroepiandrosterone.
[a]Comparison adjusted for age and cerebrospinal fluid (CSF) progesterone levels.
[b]Significant ($p < .05$) difference between posttraumatic stress disorder (PTSD) and Healthy groups.

blocks in the conversion of precursor to product steroid (see Figure 1).

Spearman correlations were performed between CSF ALLO or DHEA levels or the ALLO/DHEA ratio and: 1) clusters of related DSM-IV–defined PTSD symptoms identified by confirmatory factor analysis (Simms et al 2002) in the PTSD subjects, and 2) POMS symptoms scores in all subjects. Bonferroni corrections were used to account for the multiple hypotheses under consideration. It should be noted that Bonferroni corrections are very conservative and pose a risk for Type 2 error especially in this type of situation wherein many of the psychiatric symptoms or neuroactive steroid levels and ratios might have common mechanistic determinants and might be highly correlated.

Descriptive statistics are expressed as group means with SEM.

Results

Demographic Characteristics

Subjects' age, weight, education, trauma exposure, and past or current psychiatric diagnoses besides PTSD are tabulated in Table 1. The PTSD and healthy groups differed significantly by age but not by weight or education.

Ascertainment of Covariates

The CSF progesterone and ALLO levels were positively correlated (all subjects: $r = 48, p < 04$; healthy: $r = .77, p < 01$; PTSD: $r = .47, p = 20$). There was a trend for a negative correlation between age and CSF ALLO levels in all subjects ($r = -45, p < 06$; $n = 19$). This trend occurred in the healthy group considered alone ($r = -.60; p < 07$) but not in the PTSD group ($r = .13, p = 73$). There were no correlations between body weight and neuroactive steroid levels.

Differences in Neuroactive Steroid Levels Between PTSD and Healthy Subjects

There were no significant differences between the PTSD and healthy subjects in CSF levels of progesterone, 5α-DHP, or the progesterone/5α-DHP ratio (Table 2, Figure 2A). The CSF ALLO levels were significantly lower in the PTSD group (Table 2, Figure 2B), whereas the 5α-DHP/ALLO ratio was higher. Linear regression

adjusting for age and CSF progesterone levels also revealed group differences in CSF ALLO levels ($t = -2.77, p < .01$) and the 5α-DHP/ALLO ratio ($t = 2.34, p < .04$).

The CSF DHEA levels were not different between the PTSD and healthy groups, but the ALLO/DHEA ratio was low in the PTSD subjects (unadjusted comparison: $p < .008$; comparison adjusted for age and CSF progesterone levels: $t = -2.29, p < .03$) (Table 2B).

Follesa et al (2002) showed that oral contraceptive administration reduces progesterone and allopregnanolone levels, measured by a less-specific radioimmunoassay method, in the plasma and cerebral cortex of rats and in the plasma of women. In the current study, the PTSD and healthy groups were matched for number of oral contraceptive users. In addition, we performed statistical analyses to determine whether oral contraceptive use might have contributed to the study findings. We found no significant difference in neuroactive steroid levels or their ratios between subjects receiving oral contraception and those in the natural follicular state, when all subjects were considered together or when the PTSD and healthy groups were considered separately. Linear regression analyses controlling for use of oral contraceptives in addition to age and CSF progesterone levels revealed that group differences in CSF ALLO levels ($p < 04$) and the ALLO/DHEA ratio ($p < 02$) persisted. A trend for a group effect on the 5α-DHP/ALLO ratio was seen in this model ($p < .07$).

The Relationship Between CSF Neuroactive Steroid Levels and General Mood Symptoms

When all of the subjects were considered together, the CSF ALLO/DHEA ratio correlated strongly and negatively with the POMS depression/dejection and fatigue subscales (Table 3). When the PTSD and healthy subjects were evaluated separately, however, these correlations were preserved in the healthy ($r = -.76, p = 01$ and $r = -.72, p = .02$, respectively) but not the PTSD group ($r = 03, p = 95$ and $r = .33, p = 38$, respectively). Nevertheless, when the PTSD group was divided into five subjects with current PTSD alone and four subjects with current comorbid PTSD/MDD, a post hoc analysis revealed that CSF ALLO levels were significantly lower

Table 3. Spearman Correlations Between Neuroactive Steroid Levels and Mood/PTSD Symptoms

Correlations With Profile of Mood State (POMS) Scores in All Subjects (n = 19)			
Profile of Mood State (POMS) Scores	ALLO	DHEA	ALLO/DHEA
Total Poms	$r = -.45$	$r = .09$	$r = -.52$
	$p = .05$	$p = .71$	$p = .02$
Anger/Irritation	$r = -.43$	$r = .25$	$r = -.57$
	$p = .06$	$p = .30$	$p = .01$
Anxiety/Tension	$r = -.46$	$r = .06$	$r = -.50$
	$p = .045$	$p = .79$	$p = .03$
Confusion	$r = -.31$	$r = .16$	$r = -.43$
	$p = .20$	$p = .52$	$p = .07$
Depression/Dejection	$r = -.52$	$r = .27$	$r = -.70$
	$p = .02$	$p = .25$	$p = .0008^a$
Fatigue	$r = -.50$	$r = .30$	$r = -.63$
	$p = .03$	$p = .21$	$p = .004^b$
Vigor	$r = .23$	$r = .46$	$r = .03$
	$p = .34$	$p = .05$	$p = .90$

Correlations With PTSD Symptom Clusters in PTSD Subjects (n = 9)			
Clusters of Related DSM-IV–Defined PTSD Symptoms Identified by Confirmatory Factor Analysis (Simms et al 2002)	ALLO	DHEA	ALLO/DHEA
Re-Experiencing: Symptoms in this cluster are the same as DSM-IV Criterion B PTSD re-experiencing symptoms B1-intrusive thoughts B2-nightmares B3-flashbacks B4-emotional distress at trauma cue exposure B5-physiological reactivity to trauma cues	$r = -.72$ $p = .03$	$r = .55$ $p = .13$	$r = -.82$ $p = .007^c$
Active Avoidance C1-avoidance of trauma-related thoughts, feelings, conversions C2-avoidance of people, places, things	$r = -.08$ $p = .83$	$r = .23$ $p = .56$	$r = -.24$ $p = .53$
Dysphoria C3-amnesia C4-decreased interest in everyday activities C5-detachment or estrangement from others C6-restricted range of affect C7-sense of foreshortened future D1-disturbed sleep D2-irritability D3-poor concentration	$r = .03$ $p = .95$	$r = -.18$ $p = .65$	$r = .04$ $p = .91$
Arousal D4-hypervigilance D5-increased startle	$r = .78$ $p = .01$	$r = -.21$ $p = .59$	$r = .65$ $p = .06$

Abbreviations as in Table 2.

[a] A significant ($p < .0024$) correlation after Bonferroni correction for 21 comparisons.

[b] Trend for a significant ($.0024 < p < .0048$) correlation after Bonferroni correction for 21 comparisons.

[c] A trend ($p < .008$) for a significant correlation after Bonferroni correction for 12 comparisons.

in the PTSD/current MDD group (7.7 ± 4.6 fmol/mL vs. 19.3 ± 5.4 fmol/mL, Mann–Whitney $p = 015$).

The Relationship Between CSF Neuroactive Steroid Levels and PTSD Symptoms

In the small sample of nine PTSD subjects, there was a trend for a strong negative Spearman correlation between the CSF ALLO/DHEA ratio and PTSD re-experiencing symptoms ($r = -.82$, $p < .008$, Table 3 and Figure 3). In addition, a post hoc analysis revealed that re-experiencing symptoms were significantly higher in the PTSD/current MDD group compared with the PTSD alone group (16.2 ± 2.2 vs. 8.6 ± 5.5 fmol/mL, respectively; Mann–Whitney test $p = 04$). As noted in the previous section, the PTSD/current MDD group also had lower CSF ALLO levels.

Discussion

Summary of Findings

Women with PTSD who were free of psychotropic medications for several months or longer were found to have decreased CSF ALLO levels that were only 39% of healthy group levels, whereas CSF DHEA levels were normal. If levels of these neuroactive steroids in CSF reflect levels in brain, the data suggest that the balance between CNS inhibitory and excitatory neurosteroids might be diminished in the women with PTSD. Consistent with this possibility was a strong negative correlation between the CSF ALLO/DHEA ratio (but not ALLO levels alone in this small sample) and depressive symptoms among all subjects as well as a trend for a strong negative relationship between the ALLO/DHEA ratio and
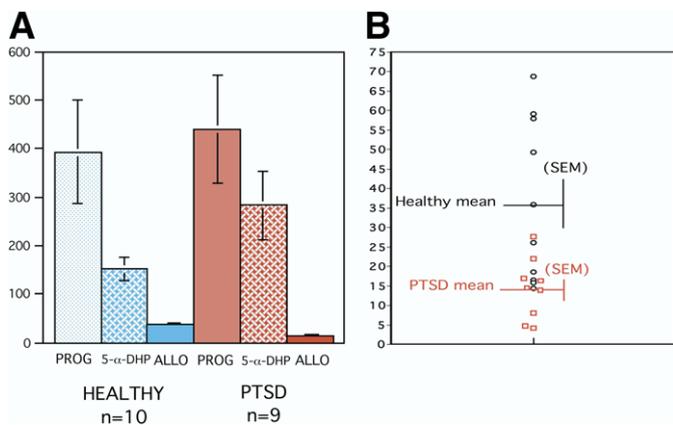
Figure 2. (A) Relative cerebrospinal fluid levels of progesterone (PROG), 5α-dihydroprogesterone (5α-DHP), and 3α5α and 3α5β-tetrahydroprogesterone (ALLO) in 9 women with posttraumatic stress disorder (PTSD) and 10 healthy nontraumatized women; **(B)** a scattergram of the CSF ALLO levels in the 10 healthy subjects (black circles) and 9 PTSD subjects (red squares).

PTSD re-experiencing symptoms among the PTSD subjects. In addition, CSF ALLO levels were lower and PTSD re-experiencing symptoms higher in the PTSD subjects with current comorbid MDD compared with the PTSD subjects without current MDD. This study thus extends previous work finding low CSF ALLO levels in male and female patients with MDD (Uzunova et al 1998) to a cohort of premenopausal women with PTSD.

Mechanisms That Might Produce Low ALLO Levels in Women With PTSD

In the only work thus far to model reductions in brain ALLO levels in response to chronic stress, Dong et al (2001) found decreased brain ALLO levels in male mice exposed to prolonged social isolation, an effect reproduced in testosterone-treated ovariectomized female mice but not in normal female mice (Pinna et al 2005). In this model, decreased brain ALLO levels resulted from downregulation of 5α-reductase gene expression and inadequate
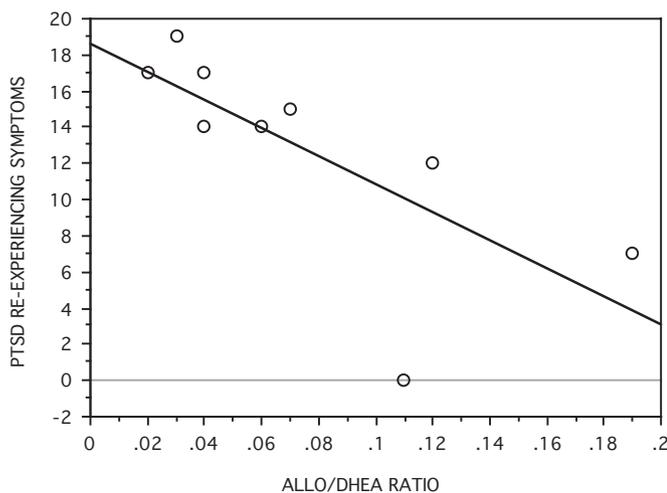

Figure 3. Relationship between posttraumatic stress disorder (PTSD) re-experiencing symptoms and the ratio of cerebrospinal fluid (CSF) 3α5α and 3α5β-tetrahydroprogesterone (ALLO) levels to CSF dehydroepiandrosterone (DHEA) levels in nine women with PTSD (Spearman $r = -.82$, $p < .008$: trend with Bonferroni corrections).
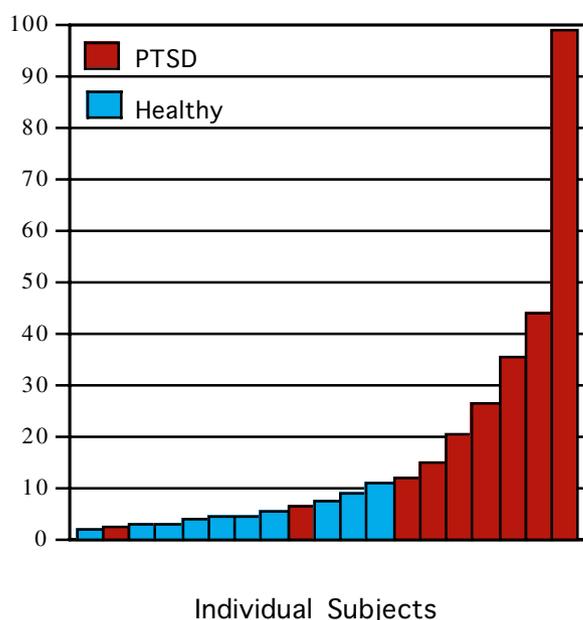

Figure 4. The ratio of 5α-dihydroprogesterone (5α-DHP) to 3α,5α and 3α,β,5β-tetrahydroprogesterone (ALLO) in the cerebrospinal fluid of individual posttraumatic stress disorder and healthy, nontraumatized premenopausal female subjects.

provision of the precursor steroid, 5α-DHP, for conversion into ALLO (see Figure 1).

The decrease in CSF ALLO levels in the women with PTSD examined in the current study cannot, however, be attributed to a downregulation of 5α-reductase levels or function. Progesterone and 5α-DHP levels as well as the ratio between progesterone and 5α-DHP were not different between the PTSD and healthy groups (Table 2 and Figure 2). Instead, the 5α-DHP/ALLO ratio was high in the women with PTSD (Figure 4). Thus, we believe that the low ALLO levels in the women with PTSD are due to either an enhancement of ALLO conversion to unmeasured inactive metabolites or a reduced conversion of 5α-DHP to ALLO. Previous work by Uzunova et al (1998) showed that levels of 3α,5α,20α−hexahydroprogesterone, one of the inactive metabolites of allopregnanolone, were below the limits of detection (< 1 fmol/mL) in the CSF of healthy and depressed subjects. Although it is possible that this metabolite could be increased in the CSF of our PTSD sample (we did not measure it), we believe that reduced conversion of 5α-DHP to ALLO is the most likely explanation for our findings.

There are several mechanisms by which conversion of 5α-DHP to ALLO could be deficient in PTSD. A combination of these mechanisms might operate within an individual, or different mechanisms might operate within different individuals. Because the enzyme that converts 5α-DHP to ALLO, 3α-hydroxysteroid dehydrogenase (3α-HSD), operates in a bidirectional manner, it is possible that faulty inhibition of 3α-HSD oxidase activity by accumulated reduced nicotinamide adenine dinucleotide phosphate (NADPH) under conditions of stress could result in preferential conversion of ALLO to 5α-DHP and deficient ALLO levels during stress (Penning et al 2004). Alternatively, a functional polymorphism or frank mutation of the 3α-HSD gene or dysregulation of 3α-HSD gene expression could lead to reduced CSF ALLO levels (Hou et al 1998).

Properties of Allopregnanolone of Possible Relevance to PTSD

In the CNS, ALLO acts in the nanomolar range as a positive allosteric modulator of GABA action at $GABA_A$ receptors (Lambert et al 2003; Puia et al 1990); ALLO also inhibits nicotinic acetylcholine receptor-mediated currents, although the behaviorally inert 3β isomer of ALLO is equipotent in this respect. Thus the anxiolytic, anticonflict, anticonvulsant, anesthetic, sedative, and neuroprotective actions of ALLO (Ciriza et al 2004; Djebaili et al 2005; Kaminski et al 2003; Rhodes et al 2004) are all believed to be a consequence of ALLO's action at $GABA_A$ receptors (Guidotti et al 2001; Pinna et al 2000; Puia et al 2003).

Recent work suggests that although neurosteroids such as ALLO have activity at all subtypes of $GABA_A$ receptors, they have highest affinity for a benzodiazepine-resistant subset of extrasynaptic $GABA_A$ receptors composed of α_4 and δ subunit combinations or α_6, γ, and β subunit combinations (Lambert et al 2003). These extrasynaptic receptors are activated by concentrations of GABA lower than that required for activation of synaptically located $GABA_A$ receptors. As a consequence, they are thought to maintain a tonic inhibitory conductance that modulates gain in neuronal output during periods of increased input (Mitchell and Silver 2003; Mody and Pearce 2004; Semyanov et al 2003, 2004). Of note, α_4, δ, and α_6 $GABA_A$ receptor subunits increase under conditions in which allopregnanolone levels are decreased (Follesa et al 2001; Gulinello et al 2002; Pinna et al 2004; Smith et al 1998; Sundstrom-Poromaa et al 2002). These putative "compensatory" receptor changes might be inadequate, however, because anxious behavior, irritability, and aggression are observed to increase under these circumstances.

We thus speculate that an uncompensated defect in the synthesis or disposition of ALLO with resultant reductions in tonic GABA-mediated inhibitory conductance might contribute to the pathophysiology of PTSD, at least in some individuals.

Low ALLO Levels and PTSD Neurobiological Phenotypes

Low CSF ALLO levels or a low ratio of ALLO to DHEA, resulting in a relative decrease in CNS inhibitory versus excitatory tone, could contribute directly to hyperreactivity of the amygdala (Ressler et al 2002; Sun et al 2004) as well as increased extrahypothalamic CRF (Patchev et al 1994) and monoaminergic responses (Goldstein et al 1996; Southwick et al 2005) during trauma exposure or exposure to trauma reminders. A decrease in extrasynaptic $GABA_A$ receptor–mediated tonic inhibition also might facilitate phasic increases in glutamatergic and GABAergic activity that could, for instance, disrupt frontal lobe-mediated inhibition of the amygdala. This might contribute to an enhancement of fear conditioning as well as resistance to extinction of fearful conditioned responses and promote the development of PTSD (Rosenkranz et al 2003; Southwick et al 2005). Because allopregnanolone is thought to provide neuroprotection, subjects with a diminished capacity for ALLO release also might be prone to stress-induced structural and functional damage of brain areas such as the hippocampus—as seen in some populations with PTSD (Bonne et al 2001; De Bellis et al 2001; Gilbertson et al 2002; Hull 2002).

Dysregulation of the HPA axis in PTSD (Rasmusson et al 2003; reviewed in Yehuda et al 2002; Young and Breslau 2004) also might be related to low CNS ALLO levels. Decreased cortisol release during stress could interfere with upregulation of the 3α-HSD gene and limit adaptive increases in ALLO production during stress (Hou et al 1997). On the other hand, ALLO release during stress provides delayed homeostatic control over activation of the HPA axis (Barbaccia et al 2001; Patchev et al 1996) and reduces expression of the

CRF and arginine vasopressin (AVP) genes in the hypothalamus (Patchev et al 1994, 1996). Thus, low ALLO levels might account for the increases in HPA axis reactivity and increases in 24-hour urinary cortisol output observed in some populations with PTSD, such as premenopausal women with comorbid lifetime or current MDD (Heim et al 2000; Lipschitz et al 2003; Rasmusson et al 2001; Young and Breslau 2004).

Study Limitations

Although the current study is methodologically rigorous and the findings are intrinsically robust and appealing from a theoretical point of view, the small sample size constitutes a significant limitation by increasing the risk for Type II error and limiting the confidence with which the findings might be generalized. The relationship between age and CSF ALLO levels and possible effects of oral contraceptives on CSF ALLO levels (Follesa et al 2002) should be investigated again in larger human populations. In addition, smoking effects on ALLO levels should be examined. The only subject in the study who smoked had the highest ALLO level in the PTSD group. Because high-dose nicotine increases brain ALLO levels in the rat (Porcu et al 2003), it is possible that heavy smoking reduces PTSD symptoms via effects on ALLO levels, accounting in part for the increased rates of high-intensity smoking in PTSD.

The current study also did not measure all neuroactive steroids that modulate $GABA_A$ receptor function. In earlier work, we attempted to measure CSF allotetrahydrodeoxy-corticosterone (THDOC: a potent positive modulator of GABA at $GABA_A$ receptors) and 3α-diol (a weak androgenic metabolite of the potent androgen, 5α-dihydrotestosterone, and a potent positive modulator of GABA at $GABA_A$ receptors), both presumably of peripheral origin. Both were below the limits of detection. Other negative modulators of $GABA_A$ receptor function such as DHEAS, which acts more potently than DHEA at both NMDA and $GABA_A$ receptors, and pregnenolone sulfate (Baulieu and Robel 1998; Park-Chung et al 1999) can and should be assessed in future studies.

We also cannot tell from the current study whether the CSF neurosteroid levels measured constitute baseline or stress levels induced by undergoing lumbar puncture or to what extent brain, spinal cord (Patte-Mensah et al 2004), or peripheral sources might have contributed to them. Previous work suggests that the adrenal gland contributes significantly to stress-induced increases in brain and, presumably, CSF ALLO levels (Barbaccia et al 1996; Purdy et al 1991; Vallee et al 2000). This assertion, however, is based on the capacity of adrenalectomy to reduce stress-related increases in brain allopregnanolone levels and does not take into account the possibility that adrenalectomy-related corticosterone reductions might disrupt DNA glucocorticoid response element-mediated maintenance of brain 3α-HSD gene transcription (Hou et al 1997). Be that as it may, future studies using continuous CSF sampling or maneuvers that markedly increase plasma levels of adrenally derived steroids before performance of lumbar puncture might help us learn to what extent peripheral neuroactive steroids contribute to CSF steroid levels in humans.

The high degree of comorbidity between PTSD and current MDD observed in the study is consistent with rates of PTSD/MDD comorbidity observed in epidemiological studies (e.g., Kessler et al 1995). This makes it difficult to know whether the low CSF ALLO levels observed in the PTSD subjects were attributable to PTSD or depression. In fact, our data suggest that CSF ALLO levels might vary continuously with depressive symptoms. Not only did we observe a strong negative correlation between depressive symptoms and the ALLO/DHEA ratio among all subjects, but CSF ALLO levels were highest in healthy subjects having no history of MDD, lower in

PTSD subjects with past but not current MDD, and lowest in PTSD subjects with current MDD. In contrast, we observed a relationship between low CSF ALLO levels and PTSD re-experiencing symptoms, only one of which is a symptom of MDD. Second, PTSD/MDD typically constitutes more severe PTSD, owing to the fact that four PTSD symptoms are also symptoms of MDD. Third, whereas a previous diagnosis of depression has been shown to predispose to PTSD, depression diagnosed for the first time after trauma exposure is almost always associated with PTSD (Breslau et al 2000). In addition, it is unclear whether subjects in previous studies of ALLO levels in MDD were assessed for trauma exposure or comorbid PTSD (Romeo et al 1998; Uzunova et al 1998).

Finally, as previously noted, animal studies have shown that brain ALLO levels increase in response to acute stress but decrease in response to the stress of chronic social isolation. The cross-sectional design of the current study, however, did not allow us to discriminate whether low ALLO levels were pre-existing (perhaps associated with MDD) and predisposed to the development of PTSD after trauma, resulted from traumatic stress exposure, or emerged as a consequence of social isolation experienced in the aftermath of trauma—possibilities that are not mutually exclusive. Prospective studies will be necessary to evaluate these possibilities. Future studies also will be necessary to determine whether low ALLO levels are diagnosis-specific or influence patterns of symptoms or severity of illness across psychiatric disorders.

Significance

The prevalence of PTSD is about 8% in the general population (Kessler et al 1995) but reaches above 15% in combat veterans (Hoge et al 2004; Kessler et al 1995; Kulka et al 1990), victims of rape and violent assault (Breslau et al 1998; Deykin and Buka 1997; Kessler et al 1995), and adolescents exposed to compound community trauma (Fitzpatrick and Boldizar 1993; Horowitz et al 1995; Lipschitz et al 2000). In addition, the prevalence of PTSD is at least twice as high among adult women compared with men when trauma exposure is controlled (Breslau et al 1998; Deykin and Buka 1997; Giaconia et al 1995). Work that advances our understanding of factors that mediate decreases in ALLO in premenopausal women with PTSD might spur the development of new methods for the prevention or targeted treatment of this prevalent, disabling, and costly disorder as well as its comorbid conditions.

For example, women with PTSD/MDD are less likely than women with PTSD alone to recover fully in response to either prolonged exposure or cognitive processing therapy (Nishith et al 2005). Perhaps raising ALLO levels in these patients would increase treatment responsiveness. For instance, serotonin selective reuptake inhibitors (SSRIs), one class of medications effective in some individuals with PTSD (Davidson et al 2004; Stein et al 2000), have been shown to increase brain levels of ALLO in animals (Pinna et al 2003) and CSF and plasma ALLO levels in humans (Griffin and Mellon 1999; Romeo et al 1998; Trauger et al 2002; Uzunova et al 1998). Interestingly, two PTSD subjects from the current study were SSRI nonresponders. These subjects had the second and third highest 5α-DHP/ALLO ratios, suggesting that more extreme blocks in the conversion of 5α-DHP to ALLO might confer SSRI treatment resistance. Perhaps the administration of synthetic ALLO-like compounds currently under development (e.g., Gulinello et al 2003; Kaminski et al 2004) or medications such as topiramate that act, in part, directly at extrasynaptic GABA$_A$ receptors (Berlant 2004; Johnson et al 2005) would benefit such individuals. The use of other medications that increase plasma ALLO levels, perhaps by other mechanisms, also might be found to be effective in PTSD or other psychiatric disorders on this basis (Genazzani et al 2003; Marx et al 2003; Schmidt et al 2005; Strous et al 2003; Wolkowitz et al 1999).

Conclusions

Future work aimed at elucidating the mechanisms responsible for low ALLO levels in women with PTSD and ascertaining the extent to which deficits in ALLO production generalize to other PTSD populations might lead to the development of new and more effective interventions for the prevention and treatment of PTSD and its comorbid disorders.

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