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BRIEF COMMUNICATION

Low pregnenolone sulphate plasma concentrations in patients with generalized social phobia

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ABSTRACT

Background. Animal studies have shown that neuroactive steroids modulate the activity of the gamma-aminobutyric acid type A/benzodiazepine receptor complex and that these steroids display anxiolytic or anxiogenic activity depending on their positive (e.g. allopregnanolone) or negative allosteric modulation (e.g. dehydroepiandrosterone sulphate) of this receptor. This study compared plasma levels of allopregnanolone, dehydroepiandrosterone sulphate and pregnenolone sulphate in healthy controls and in patients with generalized social phobia, as assessed with the Mini-International Neuropsychiatric Interview.

Methods. Plasma concentrations of allopregnanolone, pregnenolone sulphate, and dehydroepiandrosterone sulphate were measured in 12 unmedicated male patients with generalized social phobia and 12 matched healthy male volunteers.

Results. Concentrations of pregnenolone sulphate were significantly lower in patients with generalized social phobia than in healthy controls. No statistically significant differences were found for the concentrations of allopregnanolone and dehydroepiandrosterone sulphate in plasma.

Conclusions. These results are particularly interesting since we also observed lower pregnenolone sulphate concentrations in male patients suffering from generalized anxiety disorder. Their relevance to the pathophysiology of social anxiety disorder remains to be determined.

INTRODUCTION

Social phobia is characterized by chronic fear and anxiety related to social situations. The lifetime prevalence of social phobia has been described as being as high as 13·3%, with more females than males suffering from the disorder (Kessler *et al*. 1994). Benzodiazepines (BZDs) and other compounds modulating γ-aminobutyric acid (GABA) activity such as pregabalin, gabapentin (Pande *et al*. 1999) and phenelzine, which displays strong GABA transaminase inhibitor activity (Baker *et al*. 1991), are effective in the treatment of social anxiety disorder. These findings suggest a dysregulation of $GABA_A$/BZD receptor function in social anxiety disorder.

A group of neuroactive steroids (NAS) are known to modulate the activity of the $GABA_A$/BZD receptor complex (Rupprecht & Holsboer, 1999) and the concept of unique binding site(s) on the $GABA_A$/BZD receptor complex for this group of NAS has emerged, although there is not yet a satisfactory demonstration of such sites. Three major NAS involved are the progesterone metabolite allopregnanolone (ALLO), pregnenolone sulphate (PREGS), and dehydroepiandrosterone sulphate (DHEAS). The anxiolytic activity of ALLO has been well established in several models of anxiety in rats and mice (Bitran *et al*. 1993, 1995; Picazo & Fernandez-Guasti, 1995; Wieland *et al*. 1995; Reddy &

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Kulkarni, 1997), but human data are lacking. The anxiolytic activity of ALLO is mediated by its positive allosteric agonist activity at the level of the $GABA_A$/BZD receptor complex. The allosteric activity of ALLO at the $GABA_A$/BZD receptor complex enhances the flow of chloride through the ion channels of the receptor and thereby creates hyperpolarization of the postsynaptic membrane (Bitran *et al*. 1991). It is believed that DHEAS acts in the opposite manner as a negative allosteric modulator on the $GABA_A$/BZD receptor complex, thereby producing anxiogenic effects (Reddy & Kulkarni, 1997). PREGS acts as a mixed agonist/antagonist at the $GABA_A$/BZD receptor (Majewska & Schwartz, 1987) and produces an anxiolytic activity at low doses and an anxiogenic activity at high doses in animal models (Melchior & Ritzmann, 1994).

In addition to modulation of the $GABA_A$/BZD receptor function, NAS such as PREGS also induce a positive modulation of N-methyl-D-aspartate (NMDA) receptors that has been associated with anxiogenic activity (Kash *et al*. 1999; Shekhar & Keim, 2000). It can therefore be postulated that a NAS imbalance may play an important role in the pathophysiology of anxiety and more particularly social anxiety disorder.

Abnormal plasma NAS concentrations have been described in patients with major depression (Romeo *et al*. 1998), generalized anxiety disorder (GAD) (Semeniuk *et al*. 2001), and posttraumatic stress disorder (Spivak *et al*. 2000). Surprisingly, measurements of NAS concentrations thought to play a role in anxiety have not been performed in generalized social phobia patients.

The objective of this study was to determine whether patients with generalized social phobia have decreased concentrations of NAS with anxiolytic activity and/or increased levels of NAS with anxiogenic activity.

METHOD

Plasma concentrations of NAS vary with age, sex and menstrual cycle (Genazzini *et al*. 1998). In order to avoid such sources of bias, in this pilot study, we included only males and closely matched for age social anxiety disorder patients and control subjects. Twelve male subjects with social anxiety disorder and 12 healthy male controls, ranging in age from 23 to 61 were recruited by advertisements. Healthy controls and social anxiety disorder patients were matched by age within a 3 year interval. All subjects were physically healthy. Healthy controls had no lifetime Axis I psychiatric disorders with the exception of one subject with a short history of alcohol abuse 17 years prior to his inclusion in the study. Patients with social anxiety disorder did not suffer from other current Axis I disorders. Diagnosis of generalized social phobia (GSP) was confirmed with the Mini-International Neuropsychiatric Interview (Sheehan *et al*. 1998). The mean Liebowitz Social Anxiety Scale (Liebowitz, 1987) score in social anxiety disorder patients was $91{\cdot}00 \pm 17{\cdot}76$ which put them in the moderate to severe severity range. Subjects were free of any psychotropic medications for a minimum of 2 weeks prior to the study (no subjects were on fluoxetine prior to the study). None of the subjects had a score > 9 on the Raskin Depression Scale. After a complete description of the study was made to the subjects, written informed consent was obtained.

For the analysis of ALLO and PREGS, 2–3 ml of plasma was extracted three times with ethyl acetate (1:1 ratio). The residue was dissolved in methanol and analysed by high performance liquid chromatography using a C18 symmetry column (Waters) and varying the mobile phase from 20% to 80% acetonitrile. DHEAS levels were quantitated by an enzyme immunoassay (DSL, Webster) (see Semeniuk *et al*. 2001 for a more detailed description of the methods of measurement of ALLO, PREGS and DHEAS).

Results are reported as the means $\pm$ S.D. The NAS measurements between patients and controls were compared by two-tailed independent *t* tests. A value of $P < 0{\cdot}05$ was considered significant.

RESULTS

There was no significant difference between the mean concentration of ALLO in patients with social anxiety disorder and comparison subjects ($9{\cdot}23 \pm 7{\cdot}34$ ng/ml *v.* $9{\cdot}39 \pm 8{\cdot}21$ ng/ml respectively, $t_{22} = 0{\cdot}05$, $P = 0{\cdot}96$). The mean concentration of PREGS was significantly lower in patients than in comparison subjects ($2{\cdot}57 \pm 4{\cdot}18$ ng/ml

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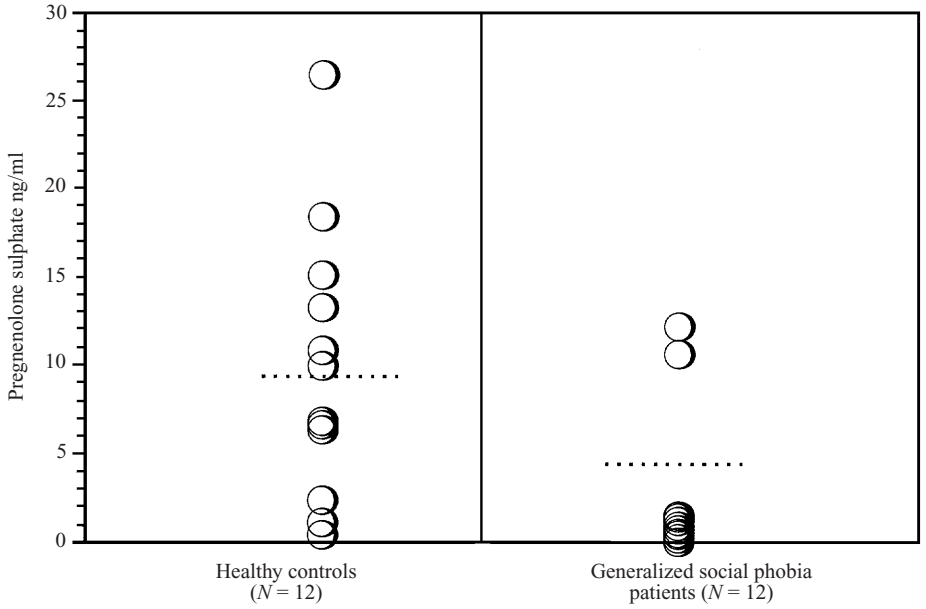


FIG. 1. Plasma levels of pregnenolone sulphate in healthy controls and in patients with generalized social phobia (...., represents the mean).

v. 9·86 ± 7·63 ng/ml respectively, $t_{22} = 2{\cdot}90$, $P = 0{\cdot}008$) (see Fig. 1). No statistically significant differences between mean level of DHEAS in social anxiety disorder patients (1772·95 ± 1425·83 ng/ml) and healthy controls (4502·65 ± 6431·08 ng/ml) ($t_{22} = 1{\cdot}43$, $P = 0{\cdot}165$) was observed. No correlation was observed between the Liebowitz scores and PREGS concentrations in social anxiety disorder patients ($r = 0{\cdot}13$, $P = 0{\cdot}69$).

DISCUSSION

We did not find any differences between patients and comparison subjects for ALLO and DHEAS. ALLO can cross the blood–brain barrier (Paul & Purdy, 1992), and consequently affect central levels. ALLO is also synthesized *de novo* in the brain (Majewska, 1992). Therefore, since we have measured only peripheral ALLO, our results do not preclude dysregulation of ALLO at the central level.

Contrary to the increased plasma concentrations of DHEAS described in male patients with post-traumatic stress disorder (Spivak *et al.* 2000), we did not observe differences between the DHEAS plasma levels of male patients with social anxiety disorder and male controls.

Our findings of lower plasma concentrations of PREGS in social anxiety disorder patients than in healthy controls are remarkably consistent qualitatively and quantitatively with the decreased plasma concentrations of PREGS that we observed in patients suffering from GAD (Semeniuk *et al.* 2001). The relevance of plasma concentrations of PREGS to central system activity is difficult to determine but the fact that PREGS crosses the blood–brain barrier (Wang *et al.* 1997) supports the clinical relevance of the observed plasma PREGS alterations. However, we did not find any correlation between PREGS plasma concentrations and the severity of the social anxiety disorder symptomatology as assessed with the Liebowitz Social Anxiety scales.

Due to this lack of correlation, the decreased PREGS concentrations are more likely to represent an insufficient compensatory mechanism. Indeed, bearing in mind that PREGS has been shown to be anxiolytic at low dose through positive allosteric modulation at the level of the $GABA_A$/BZD receptor but anxiogenic at high dose through negative allosteric modulation at the level of the $GABA_A$/BZD receptor, the observed decreased levels of PREGS could be interpreted as an attempt to cope with patho-

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logical anxiety by counteracting the detrimental antagonist activity of PREGS at the level of the $GABA_A$/BZD receptor. In addition, data from animal studies suggest that the anxiogenic activity of PREGS can also be mediated by its positive allosteric modulator activity at the level of the NMDA excitatory amino acid receptor (Wu *et al*. 1991), which is in accordance with the anxiogenic activity associated with increased glutamatergic activity (Kash *et al*. 1999; Shekhar & Keim, 2000). If we consider the lack of pathological anxiety as the result of a homeostatic balance between endogenous anxiolytic and anxiogenic agents and more particularly determined as an equilibrium between GABAergic and glutamatergic activity (Sajdyk & Shekhar, 1997), our body can maintain homeostasis through fluctuations of the intensity of GABA and glutamatergic function. In the context of pathological anxiety, decreased PREGS production could be interpreted as a homeostatic attempt to decrease anxiogenic activity through a lesser negative modulation of the $GABA_A$/BZD receptor and a lesser positive modulation of the NMDA receptor.

Plasma concentrations of NAS vary with age, sex and menstrual cycle (Genazzini *et al*. 1998). In order to avoid such sources of bias, we included only males and closely matched for age social anxiety disorder patients and control subjects. Obvious sources of variations in NAS levels cannot therefore explain our results. However, the consequence of this methodological choice is that the generalization of our results is limited. Another negative consequence of this choice was a reduction of our sample size due to our limited ability to recruit unmedicated male patients suffering from social anxiety disorder but without co-morbid Axis I disorder. The dysregulations in baseline NAS levels that we observed in social anxiety disorder patients therefore need to be replicated in a larger sample of social anxiety disorder, which include females and multiple rather than single measurements of NAS. A global interpretation of our results would also be facilitated if other NASs affecting $GABA_A$/BZD receptors, as for example tetrahydrodeoxycorticosterone ($3\alpha,5\alpha$-THDOC), are also measured.

In summary, we have found that PREGS levels are decreased in male patients with social anxiety disorder previously found in male patients with GAD. Our results suggest that anxiety disorders share some but not all dysregulations in NAS. The relevance of these dysregulations remains to be determined. Based on the efficacy of selective serotonin reuptake inhibitors in the treatment of social anxiety disorder and their ability to alter enzyme kinetics involved in NAS metabolism (Griffon & Mellon, 1999), we are currently testing whether blockade of serotonin reuptake is associated with normalization of PREGS in social anxiety disorder patients.

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