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ORIGINAL ARTICLE

Women with polycystic ovary syndrome have elevated serum concentrations of and altered $GABA_A$ receptor sensitivity to allopregnanolone

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Summary

Objective Several studies have reported that γ-aminobutyric acid (GABA) ergic circuits are involved in the pathophysiology of polycystic ovary syndrome (PCOS). The progesterone metabolite allopregnanolone is a potent $GABA_A$-receptor-modulating steroid, and patients may have increased concentrations of allopregnanolone or altered $GABA_A$ receptor sensitivity. We investigated both of these possibilities in this study.

Patients We enrolled 9 women with PCOS and 24 age-matched eumenorrhoeic controls, who were divided into two groups by body mass index (BMI) (16 normal weight and 8 overweight).

Measurements We investigated the effects of allopregnanolone injection on $GABA_A$ receptor sensitivity in both groups of women. All women received a single intravenous dose of allopregnanolone (0·050 mg/kg). $GABA_A$ receptor sensitivity was assessed with the saccadic eye velocity (SEV) over 30° (SEV30°), the SEV30°/allopregnanolone concentration ([Allo]) ratio, and sedation, which were measured together with serum allopregnanolone at intervals for 180 min after injection. The controls were tested in the follicular phase of the menstrual cycle.

Results Baseline allopregnanolone concentrations were higher in the PCOS women than in the normal-weight ($P = 0{\cdot}034$) and overweight controls ($P = 0{\cdot}004$). The allopregnanolone concentrations after injection were higher in the PCOS women ($P = 0{\cdot}006$) and overweight controls ($P = 0{\cdot}037$) than in the normal-weight controls. All groups showed a decline in the SEV30°/[Allo] ratio after injection. Allopregnanolone had a smaller effect on the SEV30°/[Allo] ratio in the overweight women (PCOS, $P = 0{\cdot}032$; controls, $P = 0{\cdot}007$) than in the normal-weight controls. The sedation score after allopregnanolone injection was lower in the PCOS patients than in the controls, but was not different between the two control groups.

Conclusions PCOS women had elevated baseline allopregnanolone concentrations compared with follicular-phase controls. All overweight women (PCOS and controls) were less sensitive to allopregnanolone than normal-weight controls.

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disturbance (6–8%) in women of fertile age. It is characterized by polycystic ovaries, amenorrhoea or oligomenorrhoea, hyperandrogenism and obesity. Obesity is a common symptom, observed in >50% of women with PCOS. Androgens and weight gain accelerate their hyperandrogenic status and insulin resistance.[1,2] Women with PCOS are considered to have elevated concentrations of the progesterone metabolite allopregnanolone (3α-hydroxy-5α-pregnane-20-one) and elevated concentrations of androstanediol (3α,17β-dihydroxy-5α-androstane), a 5α-reduced metabolite of testosterone and androstenedione (see Fig. 1).[3–5] Both allopregnanolone and androstanediol are potent positive type A γ-aminobutyric acid ($GABA_A$)-receptor-modulating steroids and have actions similar to those of benzodiazepines, barbiturates and alcohol.[6]

GABAergic circuits in the brain are involved in feeding regulation, and allopregnanolone is a strong inducer of overeating and weight gain, as demonstrated in rodents.[7] Therefore, allopregnanolone may induce obesity in PCOS women.[8] The serum concentrations of allopregnanolone are higher in obese individuals (both men and women) than in normal-weight controls.[9–11] However, the treatment of women with PCOS with metformin reduces their allopregnanolone concentrations.[5] Chronic anovulation is very common in PCOS. One reason is the chronically increased gonadotrophin-releasing hormone pulse frequency, which results in increased luteinizing hormone (LH) pulsatility, generating a higher basal LH concentration relative to the follicle-stimulating hormone (FSH) concentration.[12] There is also evidence that GABAergic circuits are involved in the increased LH pulse frequency in PCOS.[13]

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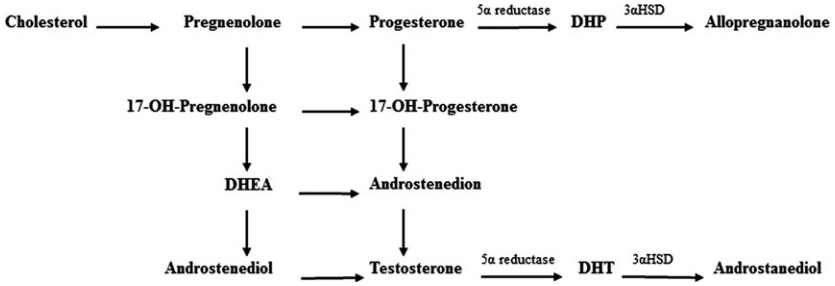

Fig. 1 Steroid synthesis pathway. DHEA, dehydroepiandrosterone; DHP, dehydroprogesterone; DHT, dihydrotestosterone; 3αHSD, 3-alpha-hydroxysteroid dehydrogenase.

Because both allopregnanolone and androstanediol are potent stimulators of GABA activity, it is not unreasonable to suspect that they are also involved in the induction of the high LH pulse frequency in PCOS. It is unknown whether elevated concentrations and/or prolonged stimulation with allopregnanolone in PCOS can cause the development of tolerance to it.[6,14] Variations in $GABA_A$ receptor sensitivity to allopregnanolone can occur in several clinical conditions. Measurement of the saccadic eye velocity (SEV) is an objective method to assess $GABA_A$ receptor sensitivity and has been used to assess its sensitivity to benzodiazepines, alcohol and pregnanolone, including in women with premenstrual syndrome[15] or people with panic disorder.[16] A saccade is a rapid, jump-like movement of the eye from one fixation point to another. Once a saccade has begun, it is beyond conscious control and not subject to motivational influence.[16–20] SEV is reduced in a dose-dependent manner by positive $GABA_A$-receptor modulators, such as benzodiazepines, allopregnanolone and alcohol.[17,21–25] An increase in self-rated sedation is another pharmacological effect of allopregnanolone and is strongly correlated with a reduction in SEV.[25]

The aims of this study were to investigate whether the sensitivity of the $GABA_A$ receptor to allopregnanolone is altered in overweight women with PCOS and whether baseline serum concentrations of allopregnanolone differ between women with PCOS and healthy controls.

Research design and methods

Healthy women aged 18–40 years were recruited through advertisement and included one group of overweight women with PCOS and two healthy control groups with regular ovulatory menstrual cycles. Twenty-six women were screened in the control group and nine in the PCOS group. All PCOS women in this study had a body mass index (BMI) $\geq$25 kg/m^2. In the control group, two subjects were excluded because their progesterone concentrations during the luteal phase did not indicate ovulation, so 24 women were ultimately included in the control group, categorized by their BMIs into two groups: 16 normal weight (BMI <25 kg/m^2) and eight overweight (BMI $\geq$25 kg/m^2). The inclusion criteria for the control group were healthy women with regular ovulatory menstrual cycles, and in the PCOS group were women with PCOS and overweight (BMI $\geq$25 kg/m^2). The diagnosis of PCOS was according to the Rotterdam Consensus 2003,[26] but more rigorous, with three of the three criteria required rather than two of three criteria: 1, biochemical and/or clinical hyperandrogenism; 2, oligomenorrhoea or amenorrhoea; and 3, at least one polycystic ovary seen on a gynaecological ultrasound examination.

The exclusion criteria in the PCOS group were other differential diagnoses of oligomenorrhoea, such as hyperprolactinaemia, hypo- or hyperthyroidism, or premature menopause, based on testing for FSH, LH, testosterone, sex-hormone-binding globulin (SHBG), dehydroepiandrosterone sulphate (DHEAS), prolactin and thyroid-stimulating hormone (TSH). The exclusion criteria in all groups were treatment with steroids (including contraceptives and hormonal intrauterine devices) in the 6 months preceding the study and treatment with psychotropic drugs (antidepressant medication, neuroleptics, benzodiazepines or other anxiolytics, or sleeping pills) within the preceding 3 months. Other exclusion criteria were any current or previous significant somatic disease or mental disorder, or a history of drug or alcohol abuse. The absence of psychiatric disorders was confirmed with a structured psychiatric interview, the Primary Care Evaluation of Mental Disorders (PRIME-MD), which has been validated for use in primary care settings and conforms to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition.[27] Women trying to become pregnant, women with eye diseases that complicated the measurement of saccadic eye movements and women with language problems were excluded. The women underwent physical and gynaecological examinations before inclusion. Women with a systolic blood pressure <90 or >170 mmHg or a diastolic blood pressure <50 or >100 mmHg were excluded. Routine blood chemistry screening (total blood cell count, plasma glucose, liver enzymes, creatinine, sodium and potassium) and a pregnancy test were performed. Women with abnormal findings on physical and gynaecological examinations, including a vaginal ultrasound, were excluded, as were those with an abnormal biochemical test result or a positive pregnancy test.

The women gave their oral and written informed consent before their inclusion in the study. The study procedures were in accordance with ethical standards for human experimentation established by the Declaration of Helsinki 1975 and revised in 1983. The Regional Ethical Review Board in Umeå, Sweden and the Medical Products Agency of Sweden approved the study.

The intravenous allopregnanolone medication was prepared at Umeå University Hospital Pharmacy, as described previously.[25] The solution was formulated with 15 mg of allopregnanolone (3α-hydroxy-5α-pregnan-20-one; Umecrine AB, Umeå, Sweden)

and dissolved in 100 ml of albumin solution (200 mg/ml) in an ultrasound bath. The concentration was determined with high-performance liquid chromatography and UV absorbance after filtration. The final solution contained 0·126 ± 0·003 mg/ml (mean ± SEM) allopregnanolone. The stability of the solution was shown to be ≥14 days, and a longer period was not investigated.

Study protocol

The women with PCOS were all anovulatory, and blood samples were taken on the SEV test day, and on days 2–5 of the menstrual cycle in the control groups. For each assay, all blood samples were analysed in the same run to reduce the impact of interassay variability. Allopregnanolone was administered once, and the PCOS women who had bleeding periods were given the allopregnanolone injection just after a spontaneous menstruation. In both control groups, the administration of allopregnanolone was performed in the follicular phase of the menstrual cycle. To avoid the possible influence of diurnal variations, all study patients were tested at the same time of the day, and the allopregnanolone injection was given between 7.50 and 8.40 AM. No subject had consumed alcohol for 24 h before testing. Caffeine and tobacco use were restricted throughout the study day. On the study day, two intravenous cannulae were inserted, one for the administration of the medication and the other in the contralateral arm to obtain blood samples. The blood used to measure the baseline serum concentrations of allopregnanolone, FSH, LH, progesterone and oestradiol was drawn 45 min before the allopregnanolone injection. One single intravenous injection of allopregnanolone (0·050 mg/kg) was given, with an infusion rate of 2 mg/min. After the allopregnanolone injection, blood samples were drawn from the contralateral arm at 5, 18, 30, 45, 60, 120 and 180 min.

The mid-luteal serum progesterone concentrations according to the sampling days were used to date ovulation in all subjects. The cycle was interpreted as an anovulatory menstrual cycle if the luteal-phase serum progesterone was <15 nmol/l. Serum progesterone was analysed with the Immulight® 1000 system (DPC, now Siemens, Aktiengesellschaft, Munich, Germany) at an accredited chemistry laboratory at Norrland University Hospital in Umeå, Sweden. The assay detection limit was 0·6 nmol/l, and the reference values were as follows: follicular phase, up to 1·72 nmol/l; and luteal phase, up to 68 nmol/l.

The method used for allopregnanolone analysis has been described in detail previously.[25] Briefly, the samples (0·4 ml) were extracted with diethyl ether (Merck KGaA, Damstadt, Germany). Allopregnanolone was separated from cross-reacting steroids by celite chromatography and was measured with a radioimmunoassay using polyclonal rabbit antiserum raised against 3α-hydroxy-20-oxo-5α-pregnan-11α-yl carboxymethyl ether coupled to bovine serum albumin, provided by R. H. Purdy (The Scripps Research Institute, La Jolla, CA, USA).[28] The sensitivity of the assay was 25 pg, the intra-assay coefficient of variation was 6·5%, and the interassay coefficient of variation was 8·5%.

SEV was measured by electro-oculography (EOG) using the Cardiff Saccade Generation and Analysis System 5 (CSGAAS5), as previously described.[29] The technique has been used in our laboratory for several years and has been described in detail in prior reports.[23, 30] SEV is not just a measure of sedation; it is an objective method of estimating sedation with small intra-individual variations.[17] Each saccade is analysed to determine the size of the saccade in degrees and the peak saccadic velocity. Saccade accuracy is determined by comparing the actual eye position at the end of the saccade with the attempted target. No data on saccadic accuracy are given in this article. The relationship between saccade size and peak velocity is important because it remains constant, even when voluntary control of the saccade is attempted. In this study, saccades and peak velocities with amplitudes of 30° were chosen for further analysis.[31] SEV was measured at baseline and at 5, 18, 30, 45, 60, 120 and 180 min after the injection of allopregnanolone.

Sedation was subjectively rated on a visual analogue scale (VAS).[32] The scale ranged from 0 to 10 cm, where 0 represented complete absence of sleepiness and 10 represented nearly falling asleep. The ratings were made at the same time as SEV was tested.

Three different scales, the State-Trait Anxiety Inventory (STAI, state subscale), the Anxiety Sensitivity Inventory and the Panic Symptom Scale (PSS), were used at baseline to measure the subject's tendency to develop anxiety and the actual anxiety level.[27–29] The difference in the anxiety level during the test was measured with the State Anxiety and Discomfort Scale (SADS) at baseline and again at 13, 25, 30, 45, 60, 120 and 180 min after the injection of allopregnanolone.[30] STAI, PSS and SADS were also used after the trial to register the anxiety symptoms or relief of anxiety in the subjects during the 24 h immediately after the test.

SPSS statistical software version 22 (IBM, Armonk, NY, USA) was used for all statistical analyses. Tests for skewness were performed to determine whether the variables had symmetrical distributions and showed that all the variables were symmetrically distributed. The SEV parameters and self-rating scores were calculated as the difference from baseline at each time point, for example ΔSEV30° (°/s). The SEV parameters after the injection of allopregnanolone were analysed with repeated-measures analysis of variance (ANOVA), and the least significant difference (LSD) test was used as the *post hoc* test.

The changes in the serum concentrations of allopregnanolone from baseline at each time point are described as means ± SEM. In each group, the changes in serum concentrations after the injection of allopregnanolone were analysed with repeated-measures ANOVA and LSD. Differences in the baseline serum allopregnanolone concentrations and the demographic data of the groups were examined with the Mann–Whitney *U*-test. *P* values of <0·05 were considered to be statistically significant.

Power analysis. Experience in our earlier trials indicated that the standard deviation of SEV among test individuals is ±7°/second (°/s).[25] The least variation in SEV between the baseline and allopregnanolone-injected samples that we wanted to detect was

15°/s, which is similar to the results of earlier allopregnanolone studies. If $\alpha = 0{\cdot}05$ and the study power = 0·90, the required size of the study population was seven test subjects per group.

Results

Among the 33 women that completed the study, 9 patients met the diagnostic criteria for PCOS. The other 24 healthy control subjects were divided into two groups according to their BMI: BMI ≥25 kg/m^2, $n = 8$ (overweight controls) and BMI <25 kg/m^2, $n = 16$ (normal-weight controls). The demographic data of the PCOS group and the controls are shown in Table 1. All the women with PCOS had oligo/amenorrhoea and anovulation, polycystic ovaries on ultrasound, clinical and biochemical signs of hyperandrogenism (hirsutism 89%, acne 33%), elevated free testosterone indices, LH/FSH ratio >2 and/or low SHBG (Table 2). All had normal TSH concentrations and no hyperprolactinaemia.

The mean baseline allopregnanolone concentrations in the PCOS group were significantly higher than those in the normal-weight controls ($P = 0{\cdot}034$) and overweight controls ($P = 0{\cdot}004$) in the follicular phase of the menstrual cycle (Table 3). BMI was, as expected, significantly higher in the PCOS group than in the normal-weight controls ($P = 0{\cdot}016$), but did not differ from that in the overweight control group. Baseline blood pressure was lower in the normal-weight controls than in the overweight controls or the PCOS group. The baseline [Allo]/BMI ratio did not differ between the groups (Table 3).

The allopregnanolone concentrations after a single injection of 0·050 mg/kg differed significantly between the groups ($F_{2,26} = 4{\cdot}62$; $P = 0{\cdot}019$). The allopregnanolone concentration was significantly higher in the PCOS group than in the normal-weight controls ($P = 0{\cdot}006$) and the overweight controls ($P = 0{\cdot}037$), whereas the allopregnanolone concentrations did not differ between the two control groups (Fig. 2).

There was no difference between the groups in the baseline SEV values. After the allopregnanolone injection, ANOVA for ΔSEV30° showed that the allopregnanolone injection had less effect on the overweight controls than on the PCOS patients ($F_{1,15} = 4{\cdot}69$; $P = 0{\cdot}047$), or normal-weight controls ($F_{1,22} = 6{\cdot}21$; $P = 0{\cdot}022$), whereas there was no difference between the PCOS patients and the normal-weight controls. However, after the effects of allopregnanolone were adjusted for the differences in its plasma concentrations, the allopregnanolone concentration was higher in the PCOS group. The ratio between SEV and the corresponding allopregnanolone concentration (SEV30° (°/s)/[Allo] (nmol/l)) was calculated for each individual woman at each time point tested. With this procedure, the SEV is related to the allopregnanolone concentration. The mean SEV/[Allo] was calculated at sampling time (Fig. 3). The change in the SEV30°/[Allo] ratio differed significantly between the study groups ($F_{2,26} = 5{\cdot}027$; $P = 0{\cdot}014$) at 45–120 min. Allopregnanolone showed greater efficacy in the normal-weight controls than in the PCOS group ($P = 0{\cdot}032$) or overweight controls ($P = 0{\cdot}007$), but there was no difference between the PCOS patients and the overweight controls (Fig. 3).

Within the PCOS group, the SEV30°/[Allo] ratio decreased over time at 0–60 min ($F_{5,40} = 4{\cdot}21$; $P = 0{\cdot}004$). In the normal-weight controls, the SEV30°/[Allo] ratio decreased over time from pre-injection to 60 min after injection ($F_{5,65} = 8391$; $P < 0{\cdot}001$). In the overweight controls, there was also a significant reduction in the SEV30°/[Allo] ratio after the injection of allopregnanolone ($F_{5,35} = 3{\cdot}81$; $P < 0{\cdot}01$) (Fig. 3).

The baseline value for sedation did not differ between the three groups (Table 3), but the subjective rating of sedation with VAS over time after the allopregnanolone injection showed significant overall differences between the groups ($F_{2,30} = 3{\cdot}73$; $P = 0{\cdot}036$). The *post hoc* tests showed that the changes in sedation in the PCOS group differed from those in both the normal-weight controls ($P = 0{\cdot}023$) and the overweight controls

Table 1. Baseline demographic characteristics of each group

	PCOS group BMI ≥25 kg/m^2 $n = 9$	Control group BMI <25 kg/m^2 $n = 16$	*P* value PCOS *vs* BMI <25 kg/m^2	Control group BMI ≥25 kg/m^2 $n = 8$	*P* value PCOS *vs* BMI ≥25 kg/m^2	*P* value BMI <25 kg/m^2 *vs* BMI ≥25 kg/m^2
Age, years	28·9 ± 1·81	25·75 ± 0·48		34·9 ± 3·41		0·038
Body mass index	31·3 ± 1·1	21·0 ± 0·42	0·016	28·9 ± 1·27	NS	0·001
Weight, kg (mean ± SEM)	84·5 ± 4·7	60·4 ± 1·92	0·027	79·7 ± 2·93	NS	0·001
Menstrual cycle length, days (mean ± SEM)	–*	28·9 ± 0·63	–	28·8 ± 0·65	–	NS
Cycle day at testing (mean ± SEM)	–*	7·75 ± 0·69	–	8·83 ± 0·91	–	NS
Systolic blood pressure, mmHg (mean ± SEM)	110 ± 2·84	103 ± 2·13	0·001	118 ± 2·83	NS	0·001
Diastolic blood pressure, mmHg (mean ± SEM)	77 ± 1·27	67·6 ± 1·47	0·046	80·6 ± 3·09	0·048	0·001
Pregnancies, median (range)	1 (0–3)	0 (0–4)	–	0 (0–4)	–	
Parity, median (range)	1 (0–2)	0 (0–2)	–	0 (0–2)	–	

*All PCOS women had menstrual dysfunction, with oligomenorrhoea or amenorrhoea. NS = not significant.

Table 2. Hormonal status at inclusion in the PCOS group ($n = 9$)

	PCOS ($n = 9$) (mean ± SEM)
FSH (IU/l)	5·3 ± 0·54
LH (IU/l)	13·6 ± 1·6
LH/FSH ratio	2·6 ± 0·23
Testosterone (nmol/l)	1·4 ± 0·084
SHBG (nmol/l)	26·0 ± 3·0
Testosterone/SHBG ratio	0·052 ± 0·004
DHEAS, (μmol/l)	6·4 ± 1·2

($P = 0·022$), whereas the two control groups did not differ. The effect of the serum allopregnanolone concentration on sedation in the groups was evaluated using the sedation/[Allo] ratio at each time point. The change in the sedation/[Allo] ratio did not differ between the three groups.

Mood rating scales were used before, during and after the test. No significant changes were seen on any of the tests within the PCOS or control groups or between the groups (data not shown).

Discussion

The sensitivity of the $GABA_A$ receptor to allopregnanolone in women with PCOS has not been examined until now. In this study, we found that its sensitivity to allopregnanolone in overweight women (PCOS and overweight controls) was lower than in normal-weight controls in the follicular phase of the menstrual cycle. This difference was observed after we adjusted the SEV measures for the differences in the serum allopregnanolone concentrations after the injection of allopregnanolone, because a larger increase in the serum allopregnanolone concentration was observed in the PCOS group than in the controls. A high BMI was the common factor in the PCOS group and overweight control group, which both displayed a reduced allopregnanolone effect. The PCOS patients also showed lower sensitivity to the sedative effect of allopregnanolone than either control groups, whereas the changes in sedation after allopregnanolone injection did not differ between the two control groups. However, no significant difference in sedation was seen between the groups when the effect was normalized for the plasma allopregnanolone concentration. We interpret these results as indicating that overweight women have developed an insensitivity to (tolerance of) allopregnanolone, an endogenous $GABA_A$-receptor-modulating steroid. The development of tolerance for an exogenous positive $GABA_A$ receptor modulator is a well-known phenomenon, especially in relation to benzodiazepine use.[33] The development of tolerance for endogenous $GABA_A$ receptor modulators is less well known. However, we know from animal studies that tolerance of allopregnanolone can be induced by long-term treatment with lower doses or by short-term exposure to higher doses.[6,14]

In our study, the PCOS patients had higher allopregnanolone concentrations than both of the control groups. This supports earlier studies that have shown that overweight women have higher baseline allopregnanolone concentrations than normal-weight women.[9] Other studies have shown that healthy overweight women also have higher allopregnanolone concentrations than normal-weight healthy controls,[11] although the difference was not significant in our study.

Our results show that both the overweight PCOS patients and the overweight controls displayed similar reductions in their sensitivity to allopregnanolone, as detected by the reduction in SEV, relative to that in the normal-weight controls. This indicates that the difference in sensitivity is more strongly related to overweight than to PCOS *per se* or to the excess androgens in the PCOS group. Therefore, it seems that a factor related to overweight promotes tolerance to allopregnanolone. One such factor could be chronic increased concentrations of allopregnanolone, which are known to occur in PCOS and obese women, although the allopregnanolone concentrations did not differ significantly between the control groups in our study.

The self-rated sedation scores after allopregnanolone injection showed higher sedation in both control groups than in the

Table 3. Mean baseline characteristics of the PCOS group and the control groups (normal weight and overweight) in the follicular phase of the menstrual cycle (mean ± SEM). Differences between the PCOS group and the controls were examined with the Mann–Whitney *U*-test

	PCOS	Control group				
	$n = 9$	BMI <25 kg/m^2 $n = 16$	P*	BMI ≥25 kg/m^2 $n = 8$	P*	BMI <25 kg/m^2 *vs* BMI ≥25 kg/m^2
[Allo] (nmol/l)	0·80 ± 0·13	0·465 ± 0·03	0·034	0·534 ± 0·11	0·004	NS
SEV30° (°/s)	444 ± 21	451 ± 12·0	NS	430 ± 17·2	NS	NS
SEV30°/[Allo] ratio	715 ± 133	1023 ± 81·1	0·039	1269 ± 384	NS	NS
Sedation (VAS, mm)	14 ± 7	19 ± 4·5	NS	18 ± 5·7	NS	NS
Sedation VAS/[Allo] ratio	19 ± 8	31 ± 9	NS	28 ± 9	NS	NS
[Allo]/BMI ratio	0·026 ± 0·004	0·020 ± 0·002	NS	0·023 ± 0·001	NS	NS

*Significance was tested between PCOS and control BMI <25 kg/m^2 or control BMI ≥25 kg/m^2.
SEV, saccadic eye velocity; VAS, visual analogue scale; BMI, body mass index; [Allo], serum allopregnanolone concentration; NS, not significant; SEM, standard error of the mean.

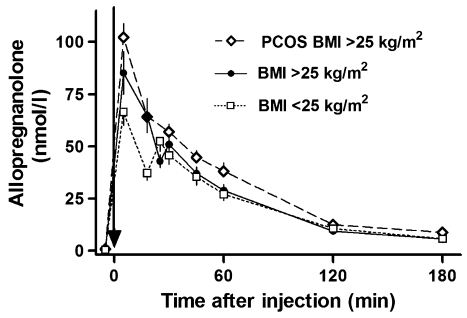

Fig. 2 Allopregnanolone serum concentrations ([Allo]) in the PCOS group and controls in the follicular phase after allopregnanolone injection. The arrow indicates when the allopregnanolone injection was given.

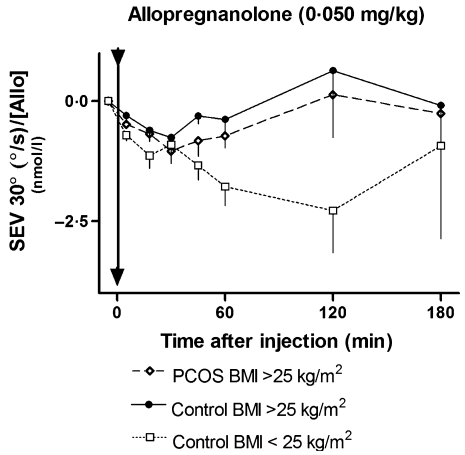

Fig. 3 Effect of the serum allopregnanolone concentration ([Allo]) on SEV30° calculated as the ratio between SEV and the corresponding [Allo] (°/s/nmol/l) for each individual woman at each time point tested.

PCOS patients. This difference could be caused by the high serum allopregnanolone concentrations in the PCOS patients, because sedation is another well-known pharmacological effect of allopregnanolone, and the difference between the PCOS patients and the controls disappeared after the sedation scores were normalized for the allopregnanolone concentration. It has been reported that SEV and sedation can change in response to positive $GABA_A$ modulators. Thyrotrophin-releasing hormone reverses benzodiazepine-induced sedation without inhibiting the SEV response.[20] Earlier reports from animal studies have demonstrated that the distribution and metabolism of allopregnanolone show region-specific effects.[34,35] We interpret these data as indicating that the effects of allopregnanolone are more pronounced in the brain areas that control SEV than in those that mediate sedation.

As well as the physiological increase in allopregnanolone concentrations during the luteal phase of the menstrual cycle and pregnancy, the serum concentrations of allopregnanolone increase during stress,[36] anxiety and panic disorders,[37] binge eating, anorexia and bulimia nervosa.[38] In the present study, the baseline serum concentrations of allopregnanolone were higher in women with PCOS than in the controls in the follicular phase of the menstrual cycle. The ratio between the baseline allopregnanolone concentration and BMI did not differ between the PCOS patients and the controls, suggesting that BMI is an important factor that affects serum allopregnanolone concentrations.

We observed differences in the pharmacokinetic properties of allopregnanolone in the PCOS women and the controls, even though the same dose (per kg bodyweight) of allopregnanolone was administered. We know that fat tissue accumulates allopregnanolone in the body.[35] If a defined amount of allopregnanolone is given intravenously, a large proportion of it will theoretically accumulate in the fat tissue, causing the serum concentration to decline after its redistribution to the fat tissue. To our surprise, we found that the serum concentrations of allopregnanolone were actually higher after a single intravenous injection in the obese PCOS group than in the controls. A possible explanation is that the rate of allopregnanolone metabolism is reduced in PCOS patients. Another explanation might be that hyperandrogenism reduces the activities of the rate-limiting steps in allopregnanolone metabolism. A further possible explanation is that the enzymatic functions involved in allopregnanolone metabolism are abnormal in women with PCOS. Because we only explored the pharmacokinetics of allopregnanolone in the peripheral compartment in this study, the differences in the allopregnanolone concentrations in the brains of women with PCOS and healthy women remain unknown. Further studies are required to investigate the pharmacokinetic properties of allopregnanolone in the brain.

In conclusion, the baseline serum concentrations of allopregnanolone were elevated in the PCOS group compared with those of control subjects in the follicular phase of the menstrual cycle. The sensitivity of the $GABA_A$ receptor to allopregnanolone was lower in women with PCOS and in overweight control women than in normal-weight controls when corrected for allopregnanolone serum concentration. The serum concentrations of allopregnanolone were significantly higher in the PCOS group after the allopregnanolone treatment. We suggest that the elimination of allopregnanolone from the circulation is delayed in women with PCOS. These findings suggest that PCOS women have a dysfunction in the GABAergic circuits. Because allopregnanolone is involved in some of the main characteristics of PCOS, (e.g. overweight and gonadotrophin secretion), the action of $GABA_A$-receptor-modulating steroids may be a new therapeutic target for the treatment of PCOS.

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