PHARMACOKINETIC AND BEHAVIORAL EFFECTS OF ALLOPREGNANOLONE IN HEALTHY WOMEN

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Short title: Behavioral effects of allopregnanolone

Abstract. *Rationale.* The behavioral effects of allopregnanolone (3α-hydroxy-5α-pregnan-20-one) in women are not known. *Objective*: Allopregnanolone, a neuroactive steroid secreted by the mammalian ovary, exerts its anesthetic, anxiolytic, and sedative/hypnotic effects through potentiation of $GABA_A$ receptors. The purpose of this study was to evaluate the behavioral effects of allopregnanolone in healthy women. *Methods*: Ten healthy women were given three increasing intravenous doses of allopregnanolone in the follicular phase of the menstrual cycle. Saccadic eye movement parameters and visual analogue scales of sedation were used to evaluate the behavioral response of allopregnanolone. Repeated blood samples for analyses of allopregnanolone were drawn throughout the study day. *Results*: Exogenously administered allopregnanolone decreases saccadic eye movement parameters and increases subjective ratings of sedation that correlate with increased serum concentrations of this neuroactive steroid. *Conclusion*: The behavioral effects of allopregnanolone are similar to that of its 5β-stereoisomer, pregnanolone (3α-hydroxy-5□-pregnan-20-one). Apart from fatigue and mild nausea, allopregnanolone given in a cumulative dose of 0.09 mg/kg did not have any adverse effects.

Key words: Allopregnanolone, neuroactive steroid, behavioral, saccadic eye velocity, menstrual cycle

Introduction

Allopregnanolone (3α-hydroxy-5α-pregnan-20-one) is an endogenous neuroactive steroid secreted by the mammalian ovary (Hodges et al. 1997; Holzbauer 1976; Ottander et al. 2005). Through binding to the $GABA_A$ receptor complexes, allopregnanolone enhances inhibitory neurotransmission (Harrison and Simmonds 1984; Majewska et al. 1986), thus exerting anxiolytic (Bitran et al. 1991, 1995; Wieland et al. 1991), sedative (Lancel et al. 1997; Sundstrom et al. 1998), and antiepileptic effects (Landgren et al. 1998). There is also evidence that allopregnanolone may mediate the effects of alcohol and benzodiazepines in both experimental animals (Morrow et al. 1999, 2001) and humans (Torres and Ortega 2003; however, see Nyberg et al. 2005). The binding site(s) of allopregnanolone to the $GABA_A$ receptor is unknown but differs from those of benzodiazepines, barbiturates, and picrotoxin (Gee et al. 1995; Lan et al. 1991).

Although allopregnanolone can be synthesized de novo in the central nervous system (CNS) from cholesterol (Stoffel-Wagner 2001), it is conceivable that plasma allopregnanolone in women predominantly originates from the corpus luteum (Backstrom et al. 1986; Ottander et al. 2005). Plasma concentrations of allopregnanolone temporally follow those of progesterone, and levels of approximately 6–10 nmol L−1 have been found in the mid-luteal phase of the menstrual cycle (Genazzani et al. 1998; Wang et al. 1996), and during third-trimester pregnancy, allopregnanolone levels up to 150 nmol L−1 have been found in maternal blood and umbilical cord blood (Hill et al. 2000, 2001; Luisi et al. 2000; Parizek et al. 2005).

Oral administration of micronized progesterone, which results in variable serum levels of allopregnanolone (Freeman et al. 1993; de Lignieres et al. 1995), is unable to relieve symptoms of anxiety and depression in women with severe premenstrual syndrome (Vanselow et al. 1996; Wyatt et al. 2001). In cognitive studies, such a single dose in those women, where high serum levels of allopregnanolone were obtained, produced increased fatigue, confusion, and poor concentration, symptoms similar to those encountered among women with premenstrual dysphoric disorder (Freeman et al. 1993). For postmenopausal women treated with vaginal progesterone, negative mood symptoms occurred at serum concentrations of allopregnanolone similar to endogenous luteal phase levels, whereas lower or higher concentrations had no significant effect on mood (Andreen et al. 2005). Although the role of allopregnanolone thus far is unclear, numerous studies have reported altered peripheral levels of allopregnanolone in psychiatric mood disorders such as premenstrual

dysphoric disorder (Bicikova et al. 1998; Girdler et al. 2001; Monteleone et al. 2000; Rapkin et al. 1997; however, see Schmidt et al. 1994; Sundstrom and Backstrom 1998), major depression (Romeo et al. 1998), and anxiety disorders (Strohle et al. 2002).

Prior studies in laboratory animals have indicated that acute administration of allopregnanolone is anxiolytic and soporific (Bitran et al. 1991, 1999; Lancel et al. 1997; Wieland et al. 1991). However, whether allopregnanolone is intrinsically rewarding or aversive is thus far unclear, since existing evidence is contradictory. For example, in low doses, allopregnanolone induces conditioned place aversion in male rats, indicating innate negative reinforcing effects (Beauchamp et al. 2000), whereas a conditioned preference was evident at higher doses of allopregnanolone in male mice, indicating positive reinforcing effects (Finn et al. 1997). Furthermore, while it is established that acute injections of progesterone and allopregnanolone are reliably anxiolytic within an hour, a few days of high neurosteroid levels can increase anxiety and modulate $GABA_A$ receptor subunit expression and function (Friedman et al. 1993; Gulinello et al. 2001; Yu et al. 1996a, b).

The sedative effect of allopregnanolone can be evaluated by use of saccadic eye movement (SEM) measurements. A saccade is a rapid, jump-like movement of the eye from one fixation point to another, used by the eye in order to change the focus of the fovea. Maximal saccadic eye velocity (SEV) has a large variation of 350–600 deg/s between subjects (Hommer et al. 1986; Sundstrom and Backstrom 1998), but is stable within subjects, both within a testing period and between testings (Gentles and Thomas 1971; Glue et al. 1991; Hommer et al. 1986; Roy-Byrne et al. 1990; Sundstrom and Backstrom 1998). Once a saccade has started, it is generally believed to be outside conscious control and not subjected to motivational influences (Gentles and Thomas 1971), and therefore, SEV is considered to provide an objective and sensitive measure of sedation. SEV has previously been shown to be reduced in a dose-dependent manner by benzodiazepines, pregnanolone (the 5β-stereoisomer of allopregnanolone, 3α-hydroxy-5β-pregnan-20-one), and alcohol (Hommer et al. 1986; Nyberg et al. 2004; Sundstrom et al. 1997, 1998). Furthermore, benzodiazepine- and pregnanolone-induced increases in self-ratings of sedation, which is another pharmacological action of these compounds, are highly correlated with reduction in SEV (Hommer et al. 1986; Sundstrom et al. 1998).

Knowledge of allopregnanolone's effects in humans is very limited. Since allopregnanolone appears to be involved in a number of mood disorders in women, further studies of its effects need to be undertaken. The aim of the current study was to determine the pharmacokinetics of allopregnanolone and its effect on saccadic eye movement parameters and self-rated sedation

in healthy women. A secondary aim of the study was to evaluate a new egg-yolk antisera for allopregnanolone analyses.

Materials and methods

Subjects

Thirteen healthy women between the ages of 18 and 40, with regular menstrual cycles (29.4±0.8 days), were screened for inclusion in the study. They were recruited through advertisement in the local newspaper. Of these, 10 women were selected. The exclusion criteria were treatment with any steroid compound (including oral contraceptives and hormonal intrauterine devices) for at least 6 months prior to enrollment in the study, treatment with benzodiazepines or other psychotropic drugs within the last 3 months preceding inclusion, and treatment with any daily over-the counter drug during the last 4 weeks before inclusion. Women planning to become pregnant were excluded. Further exclusion criteria were any current or previous somatic disease, any mental disorder, including PMDD, during the last 6 months, or a history of drug abuse. The presence of psychiatric disorders was evaluated using a structured psychiatric interview, Primary Care Evaluation of Mental Disorders, which has been validated for use in primary care settings and conforms to the criteria in Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (Spitzer et al. 1994). Before inclusion, physical and gynecological examinations were performed, as well as routine urine and blood chemistry screens. All subjects had negative pregnancy tests and normal blood chemistry screens. No night work or jet-lag travels were allowed during the week before the study day. The women gave written informed consent prior to inclusion in the study. The study procedures were in accordance with ethical standards for human experimentation, established by the Declaration of Helsinki of 1975, revised in 1983. The Research Ethics Committee, University of Umeå, and the Medical Products Agency of Sweden approved the study.

Study protocol

Testing was carried out in a gynecological outpatient department. Allopregnanolone was administered in the follicular phase (days 5–10 of the menstrual cycle). No subjects consumed alcohol 24 h prior to testing. Caffeine and tobacco use was restricted throughout

the study day. Subjects arrived at 8:00 a.m. An intravenous cannula was inserted in each forearm, and blood samples were taken for baseline levels of allopregnanolone in serum. To establish baseline, three sets of SEV measurements and visual analogue ratings of sedation and intoxication were made, with 5-min rest in between. Thereafter, at 9:00 p.m., three intravenous injections of allopregnanolone were given at 30-min intervals, using doses of 0.015, 0.03, and 0.045 mg/ kg, thus giving a cumulative dose of 0.09 mg/kg. Each injection was given more than 30 s. The Umeå University Hospital Pharmacy prepared the experimental medications. Intravenous allopregnanolone solution was formulated with purified allopregnanolone, UC1009 (Umecrine AB, Box 7984, 907 19 Umeå, Sweden) 15 mg dissolved in 100 ml albumin solution (Pharmacia, Stockholm, Sweden, 200 mg/ml) using an ultrasound bath. The solution contained 0.126±0.003 mg/ml (mean±SEM) allopregnanolone (n=9). The allopregnanolone concentration of each batch of solution was determined using HPLC and UV absorbance (Turkmen et al. 2004). After each allopregnanolone injection, SEV recordings and visual analogue ratings were made at 5, 13, and 21 min, respectively. At the time of SEV measurements, plasma for measuring allopregnanolone levels was drawn from the arm contralateral to that used for drug administration. Additional SEV recordings and blood sampling were performed at 95, 105, 115, 150, and 330 min. Subjects were allowed to ambulate in the gynecologic ward and the hospital area after the 150-min SEV recording and blood sampling.

Blood samples were also drawn at 600 and 780 min (the last blood sample 13 h after the first allopregnanolone injection, at 10:00 p.m.).

Saccadic eye movements

Saccadic eye velocity (SEV) was measured using electrooculography (EOG) with the CSGAAS5 system, fully documented elsewhere (Marshall et al. 1985; Marshall and Richens 1989). The test was performed in a quiet, semi-lit room with the patient sitting in a comfortable chair. Head movements were prevented by supporting the subject's head with a pillow. EEG cup electrodes (Synetics AB, Stockholm, Sweden) with a small amount of electrode gel (Elefix, Nihon Kohden) were used. After the skin had been exfoliated with Skinpure cream (Nihon Kohden), the electrodes were placed 1 cm lateral of the outer canthus of both eyes, with one common electrode in the center of the forehead. Electrode impedance was measured and confirmed to be less than 5 kΩ. The subject was instructed to watch an array of light-emitting diodes (LEDs) placed at eye-level, 67 cm from the glabella. The target

for the eye movements was an illuminated LED. The subject was asked to look at the illuminated LED and to move her eyes to the next target (the next illuminated LED), as that LED was turned off, and the next one in the array was lit. Subjects were instructed not to anticipate targets.

The target movements took place at 1.5-s intervals. A fixed, nonrandom sequence of 4×24 targets producing target steps of 10°, 20°, 30, and 40° was displayed with a brief rest in between. The first four of these 24 target steps of each session were not included in the subsequent analyses in order to allow the subject to adjust to the procedure. The EOG was DC amplified and low-pass filtered (−3 dB at 50 Hz) before being digitized to 12-bit resolution at a sampling frequency of 250 Hz. A personal computer controlled the target movements and digitized the waveform using an analogue-digital converter. The 80 individual EOGs, resulting from the 4×20 target steps, were stored and analyzed off-line according to the method of Marshall and Richens (1989). First, the digitized data from each target displacement were processed to locate saccades. To avoid preemptive saccades and blinking artifacts, only saccades initiated 50 to 400 ms after target movements were included. Also, to be considered a saccade, the recorded eye movement had to display a velocity of more than 100 deg/s. Second, each saccade was analyzed to determine the size of the saccade in degrees, the peak saccadic velocity, and latency from target movement to onset of saccade. Saccade accuracy was determined by comparing the actual eye position at the end of the saccade with the attempted target. SEV was further processed by plotting a velocity–saccade size curve, known as the main sequence (Baloh et al. 1975). The relationship between saccade size and peak velocity is important since it remains constant even when voluntary control of saccades is attempted. The main sequence was fitted by a quadratic equation to the peak velocity data using the calculated saccade angle as the independent variable. The influence of outliers in the data was minimized by carrying out the fitting procedure twice and weighing the second fit with the inverse of the square of the residuals from the first fit.

The values of peak velocity for 10°, 20°, 30° and 40° saccades were then calculated by interpolation. Saccades with amplitudes of 30° were chosen for further analyses as SEV reaches a maximum at approximately 30–35° of angular movement (Baloh et al. 1975).

Visual analogue ratings

A visual analogue scale (VAS) was used to rate sedation and subjective feelings of alcohol-like intoxication (McCormack et al. 1988). The scale measured from 0 to 10 cm, where 0 equaled complete absence of sleepiness/feelings of intoxication and 10 represented falling

asleep/feelings of heavy intoxication. Subjective ratings of sedation and intoxication were made at baseline, after each of the saccadic eye measurements, and at 600 and 780 min.

Assays of Allopregnanolone

Extraction

Allopregnanolone was measured by radioimmunoassay (RIA) after diethylether extraction and celite chromatographic purification of samples. Serum or plasma (0.2–0.4 mL) was pipetted into a cylindrical flat bottom glass vial of 20 mL volume, whereafter water (0.5 mL) and diethyl ether (3.0 mL) were added. The samples were then allowed to stand on an orbital shaker for 10 min. Following the liquid–liquid extraction, the vials were transferred into an ethanol/dry ice bath. The water phase was frozen, and the ether phase was decanted and evaporated under a stream of nitrogen gas.

Celite chromatography

The evaporated sample was dissolved in 1.0 ml isooctane (Merck) saturated with ethylene glycol (J.T. Baker), before application to the column. Celite column chromatography was performed as follows. Glass columns (50 mm×5 mm i.d.) were tightly packed with a mixture of Celite (Mansville,Denver, CO, USA, heated to 600°C overnight) and propylene glycol (Merck), w/v, 1/1. Isooctane (10 ml) was percolated through the columns before sample applications. The sample was applied, followed successively by a 1.0-ml isooctane wash, 1.5 ml isooctane to elute 5α- and 5β-dihydroprogesterone, 4 ml isooctane to elute progesterone, an additional 4 ml isooctane to elute allopregnanolone, and in the next 4.0 ml isooctane to elute 3α-hydroxy-pregn-4-en-20-one and pregnanolone. 20β-Hydroxy-5α-pregnan-3-one, which cross-reacts with the rabbit antisera, is not eluted with isooctane.

The allopregnanolone-containing fraction was evaporated under nitrogen. Recovery was determined for each assay using 300–500 cpm of tritium-labeled allopregnanolone, [9,11,12-3H(N)]-5α-pregnan-3α-ol-20-one (Perkin-Elmer Life Sciences, Boston, USA) added to a plasma sample before extraction and by measuring the amount recovered after chromatography. The recovery of allopregnanolone averaged 78%, and the results are compensated for recovery.

Radioimmunoassay

All samples were analyzed using a polyclonal rabbit antiserum (Purdy et al. 1990). For the evaluation of the new egg-yolk antisera (Agrisera AB, Vannas, Sweden), some of the samples were analyzed using both the rabbit antisera and the egg-yolk antisera, n=77. Additionally, for evaluation purposes, both antisera were used to analyze 18 follicular phase samples (8 samples from the current study and 10 samples from healthy controls, used as internal standard).

The Agrisera antibody is an egg-yolk IgY antibody, immunized in the hen. Both these antisera were raised against 3α-hydroxy-20-oxo-5α-pregnan-11-yl carboxymethyl ether coupled to bovine serum albumin, (Purdy et al. 1990). The cross-reactivity of the antibodies is shown in Table 1. The standard curve was established by preparing duplicate tubes containing eight concentrations of unlabeled allopregnanolone to give a range from 0 to 5,000 pg. Rabbit antiserum was used in a dilution of 1/5,000, while the egg-yoke IgY antibody was used in a dilution of 1/1,000; otherwise, the antibody solutions were prepared in the same way as described below.

The antibody solutions were prepared using [11,12]3Hallopregnanolone, 3×106 cpm/32 ml solution containing 65 mM boric acid (Merck) buffer, pH=8.0, bovine serum albumin 100 mg/ml (Sigma, St Louis, USA), human gamma globulin solution 20 mg/ml (Octapharma, Sweden) and antibody in milliliter ratio, hen antibody solution: 30:1:1:0.032, rabbit antisera solution: 30:1:1:0.006. The solution was allowed to equilibrate overnight at 8°C. Antibody solution (200 µl) was added to all standard and sample tubes, and the mixture allowed to stand overnight at 8°C. After the addition of 200 µl saturated ammonium sulfate, each tube was again mixed and centrifuged at 20,000 RPM for 20 min. Thereafter, the supernatant was aliquoted into a counting vial and diluted with 3.0 ml Optiphase scintillation medium (Wallac, Finland). The samples were counted in a RackBeta (Wallac) scintillation counter. The sensitivity of the assays was 25 pg, with an intraassay coefficient of variation for allopregnanolone of 6.5% and an interassay coefficient of variation of 8.5%.

Pharmacokinetic and statistical analyses

Before the pharmacokinetic calculations were carried out, the baseline allopregnanolone concentration (C0) was subtracted from the measured values obtained 5–780 min later. Only the net concentrations were used for the pharmacokinetic analyses. Baseline concentrations were less than 1% of the maximum concentration at the same sampling occasion. Pharmacokinetic parameters were calculated by means of the Kinetica version 4.3 program

(InnaPhase Corporation, Philadelphia, PA, USA), using a two-compartment model. The parameter estimates describing the elimination phase (terminal phase) slopes of the log-concentration of allopregnanolone (λz) were calculated using the concentrations from 330 to 780 min after the first dosage, as this always gave the best-fit regression lines. The parameter estimates describing the distribution phase slopes of the log-concentration of allopregnanolone (λ1) were calculated using the best-fit regression line with more than three concentration/time observations included, counted backwards from the measurement after 150 min. Half-lives in the distribution phase (t1/2,1) and the elimination phase (t1/2,z) were calculated as ln2/λ1 and ln2/λz, respectively. Areas under the curve (AUC) were calculated using a mixed log-linear method with extrapolation to infinity.

The mean extrapolated AUC was 5.2% (range 0.6–10.3%) of the total AUC. Clearances (CL) were calculated as dose/AUC. The volumes of distribution in the elimination phase (Vz) were calculated as CL/λz. The volumes of distribution at steady state (Vss) were calculated as Dose×MRT/AUC. In this equation, MRT is mean residence time and is calculated as AUMC/AUC, in which AUMC is the area under the concentration–time product vs time curve from zero to infinity.

Saccadic eye movement parameters and self-rating scores were calculated as delta scores (difference from baseline at each time-point). The saccadic eye movement parameters were analyzed by one-way ANOVA (analysis of variance) with repeated measures using time-point as within-subjects factor. Post hoc tests for each time-point were obtained by Tukey Honestly Significance Test. Correlations between allopregnanolone levels, the saccadic eye movement parameters, and the subjective scores were made by partial correlation. The SPSS statistical package was used for the analyses. P values of less than 0.05 were considered to be statistically significant.

Results

Demographic data of the study group are given in Table 2. For technical reasons, saccadic eye movements after the third injection and throughout the remaining test session could not be assessed in two women. Saccadic eye movements are missing for three subjects at the 330-min measurements. Pharmacokinetic parameters for allopregnanolone are summarized in Table 3. The serum allopregnanolone concentration–time relationships are shown in Fig. 1.

Allopregnanolone induces a significant reduction in SEV ($F(14,9)=10.09$, $p<0.001$), saccade acceleration ($F(14,9)=8.15$, $p<0.001$), as well as a significant change in saccade deceleration

($F(14,9)=10.67$, $p<0.001$) and self-rated scores of sedation ($F(16,9)=11.61$, $p<0.001$) (Fig. 2). Saccade accuracy ($F(14,9)=3.25$, $p<0.001$) was also significantly deteriorated, whereas saccade latency ($F(14,9)=1.68$) was unaffected by the allopregnanolone injections (Fig. 2). According to the post hoc analyses, significant responses in SEV, saccade acceleration, and saccade deceleration were noted after the 0.03 mg/kg dose of allopregnanolone, whereas saccade accuracy and sedation scores were not affected until the 0.045 mg/kg dose. The effect of allopregnanolone on SEV, saccade acceleration, and saccade deceleration lasted 45 min after the final dose (Fig. 2). Saccade accuracy and self-rated sedation were affected until 55 min after the 0.045 mg/kg dose, Fig. 2. The maximum decrease in SEV following allopregnanolone injections was 13.2%±2.7 (mean±SEM).

Allopregnanolone serum concentrations were significantly correlated with SEV ($r=-0.48$, $p<0.001$), saccade acceleration ($r=-0.44$, $p<0.001$), saccade deceleration ($r= -0.50$, $p<0.001$), saccade accuracy ($r=-0.41$, $p<0.001$), and subjective scores of sedation ($r=-0.45$, $p<0.001$).Three women reported mild feelings of alcohol-like intoxication during the test session. Three women experienced mild nausea following the allopregnanolone injections and one woman reported flushing. Twenty-four hours after the administration of allopregnanolone, one woman reported an anxiety attack. Four of the subjects were tobacco users. Although the tobacco users were abstaining from nicotine during the test session, there was no difference in saccadic eye movement response or subjective response to allopregnanolone between these subjects and the remaining group (data not shown). However, three of four tobacco users reported adverse effects during and after the test session, whereas three of six nonusers reported any side effects.

Allopregnanolone concentrations (nmol L^{-1}) were measured using the two different antibodies in 77 serum samples. After celite chromatography separation, the serum samples were assayed with both rabbit antisera (Purdy et al. 1990) and a hen IgY antibody (Agrisera). The result of the analysis of allopregnanolone, using these two antibodies, is shown in Fig. 3. The correlation coefficient was $r=0.962$, $p<0.001$. The baseline concentrations obtained during the follicular phase of the menstrual cycle ($n=18$, mean±SEM) were 0.429±0.031 nmol L^{-1} (rabbit antisera) and 0.449± 0.039 nmol L^{-1} (egg-yolk antisera). The baseline follicular phase values were not different between the two antisera.

Discussion

The main finding of the present study is that exogenously administered allopregnanolone decreases saccadic eye movement parameters and increases subjective ratings of sedation that correlate with increased serum concentrations of this neuroactive steroid.

Prior studies with exogenous allopregnanolone administration in humans are limited. In one study, allopregnanolone was given as a vaginal gel in a dose of 90 mg to13 postmenopausal women on oral estrogen therapy. Adverse effects were few (mainly mastalgia) and interpreted as related to estradiol administration (Navarro et al. 2003). According to endometrial biopsies, their data suggested that allopregnanolone had no secretory action on the endometrium; however, 5 of 13 patients reported bleeding after the allopregnanolone addition to estradiol treatment. It should be noted that allopregnanolone is rapidly metabolized to 5α-pregnane-3,20-dione, which is a potent progestin (Milewich et al. 1979), which in turn could explain the endometrial shedding. Pregnanolone, the 5β-epimer of allopregnanolone, which is not metabolized to an active progestin, has previously been investigated in humans, predominantly for the induction of anesthesia (Carl et al. 1990, 1994). The sedative effects of pregnanolone have been studied across different hormonal conditions, such as the menstrual cycle and during hormone replacement therapy (Sundstrom et al. 1998; Wihlback et al. 2001, 2005), and depend on endogenous concentrations of neuroactive steroids. Pharmacokinetics of pregnanolone, however, appear not to be influenced by the hormonal changes during the menstrual cycle (Sundstrom et al. 1999). Pregnanolone and allopregnanolone have similar pharmacokinetic and behavioral properties in rats, although allopregnanolone appears to be more potent in some studies (Norberg et al. 1987; Wang et al. 1995; Zhu et al. 2001).

The current study demonstrates that allopregnanolone has a distribution half-life of 43.9 min, and a clearance volume of 32.6 ml min−1 kg−1. This finding might indicate that allopregnanolone has a slightly longer distribution half-life than the previously reported half-life of the pregnanolone-albumin solution (31.8 min) (Sundstrom et al.1999). Also, the clearance volume was smaller compared to that previously reported for pregnanolone (61.0 ml min−1 kg−1) (Sundstrom et al. 1999). Presumably, this could suggest that allopregnanolone is redistributed and metabolized at slower rates than pregnanolone. However, direct comparative studies are needed to verify possible differences in pharmacokinetic properties between the two stereoisomers.

It has been demonstrated in this study that allopregnanolone given intravenously in a cumulative dose of 0.09 mg/kg significantly affects parameters of saccadic eye movements, as objective measures of sedation and self-reported sedation. We have previously used a

cumulative dose of 0.18 mg/kg pregnanolone in a similar study to evaluate the sedative effects of the compound. In the present study, the 0.09 mg/kg dose of allopregnanolone induced a maximum percent decrease in SEV of 13.2%. The maximum percent decrease in SEV following a cumulative dose of 0.18 mg/kg of pregnanolone was approximately 21% (Sundstrom et al. 1998). Our findings could indicate that allopregnanolone might be more potent than pregnanolone in humans, which would be consistent with those animal studies suggesting that allopregnanolone is more potent than its 5β-stereoisomer (Norberg et al. 1987; Wang et al. 1995; Zhu et al. 2001).

The cumulative dose in this study, 0.09 mg/kg of allopregnanolone, resulted in a maximum serum concentration of approximately 70 nmol L−1. This concentration is similar to that found during third-trimester pregnancy (Hill et al. 2000, 2001; Luisi et al. 2000; Parizek et al. 2005); it produced significant effects in objective and subjective measures of sedation. Given the pronounced effect of acutely administered allopregnanolone found in this study, it is conceivable that a gradual tolerance to the sedative effects of allopregnanolone occurs during pregnancy. This hypothesis is supported by the finding that acute tolerance to the anesthetic effect of allopregnanolone in male rats, evaluated using the silent second threshold to anesthesia, rapidly develops during the course of the experiment (Zhu et al. 2004). Again, the hypothesis of a gradual development of tolerance to allopregnanolone during pregnancy needs to be evaluated in detail before any definitive conclusions can be drawn.

Adverse effects of allopregnanolone in the present study were usually mild, including three subjects reporting nausea, three subjects reporting feelings of intoxication, and one subject reporting flushing following the injection. However, 24 h following the allopregnanolone injection, one subject reported an anxiety attack, which lasted for a couple of hours. Some of the adverse effects could be due to the fact that four of the subjects during the test session were abstaining from their regular tobacco use. The behavioural and progestational responses, as well as safety aspects, of higher doses of allopregnanolone remain to be evaluated.

There are a number of limitations of the current study; this is why the study results must be interpreted with caution. The major limitation is that no control group was included in the study, whereby possible effects of general fatigue during the session cannot be distinguished from the allopregnanolone effect. However, the parameters most likely to be affected by this limitation are the subjective measures. By plotting main sequences of SEVand saccadic deceleration, we are able to control for voluntary attempts to slow down the saccadic velocity and saccade deceleration. Furthermore, SEV is considered stable within subjects both within

a testing session and between sessions, and learning effects appear not to occur (Hommer et al. 1986; Sundström et al. 1998; Sundström and Backstrom 1998).

Another potential limitation of the present study for the interpretation of the behavioral effects of allopregnanolone is that endogenous progestin levels of 5α-pregnanedione (the principal metabolite of allopregnanolone) might interfere with the behavioral variables. However, fluctuations in the endogenous levels of 5α-pregnanedione and progesterone are only likely to minimally influence the allopregnanolone concentrations in our subjects, as tests were scheduled in the early follicular phase. Furthermore, a limitation for the interpretation of pharmacokinetic effects of allopregnanolone was that blood sampling during the initial phase of the experiment was too sparse to allow a three-compartment modeling for pharmacokinetics of allopregnanolone.

The new antibody produced in the hen can adequately measure allopregnanolone concentrations after its intravenous administration. This antibody has a slightly different cross-reactivity compared to the polyclonal antibody produced in the rabbit (Purdy et al. 1990). The antigen for both antisera was the BSA conjugate of the same hapten. The hen antibody has a higher cross-reactivity for 3β-hydroxy-5α-pregnan-20-one, pregnenolone, and pregnanolone, but a lower cross-reactivity for 3α-hydroxypregn-4-en-20-one, 5β-pregnane-3,20-dione and 20β-hydroxy-5α-pregnan-3-one. The cross-reactivity of the hen antibody indicates that a preceding chromatographic step is necessary. In Materials and methods, we describe an effective chromatographic procedure, and in this study, we compared the results by RIA with both antisera. As can be seen in Fig. 1, there is a good correlation between the concentrations from the two assays. The regression line is very close to ideal and passes the y-axis close to the origin. Also, the allopregnanolone serum concentrations obtained with both antibodies give similar results.

The baseline follicular-phase allopregnanolone serum concentrations obtained in this study are similar or slightly lower compared to earlier reported concentrations by the use of chromatography and RIA (Bicikova et al. 2000; Genazzani et al. 1998; Monteleone et al. 2000; Schmidt et al. 1994; Wang et al. 1996), as well as by the use of gas chromatography/mass spectrometry (GC/MS) (Epperson et al. 2002; Strohle et al. 2002). The concentrations in the present paper are, however, higher than the concentrations reported by Pearson Murphy and Allison (2000), where very low levels of allopregnanolone were found in both the follicular and luteal phases. The reason for this discrepancy may be due to their very low recovery (mean 35%) after extraction and HPLC, which may not have been fully compensated.

In conclusion, the present study demonstrates that allopregnanolone can safely be given intravenously in low doses to women. Its behavioral effects are similar to those previously reported for pregnanolone. Further studies on the effects of and the sensitivity to allopregnanolone in subjects with psychiatric and hormonal disorders are crucial for the understanding of the neuroactive role of allopregnanolone.

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Table 1

Cross-reactivity test of IgY antibody from HEN1102-batch1219, titer 1/1000. as compared is to the cross-reactivity pattern from rabbit antisera obtained using the same hapten, 3α-hydroxy-20-oxo-5α-pregnan-11-yl carboxymethyl ether, coupled with bovine serum albumin (Purdy et al. 1990).

	Cross reactivity	
Neuroactive Steroid	Hen antibody (Agrisera)	Rabbit antisera (Purdy et al. 1990)
3α-hydroxy-5α-pregnan-20one	100%	100%
5α-pregnane-3,20-dione	62%	50%
3β-hydroxy-5α-pregnan-20-one	35%	8.3%
3α-hydroxypregn-4-en-20-one	32%	50%
pregnenolone	31%	4.0%
progesterone	17%	16.7%
3α-hydroxy-5β-pregnan-20-one	16%	5.8%
5β-pregnane-3,20-dione	12%	21.3%
20β-hydroxy-5α-pregnan-3-one	<1%	13.5%
3β-hydroxy-5β-pregnan-20-one	<1%	<1%
20α-hydroxypregn-4-en-3-one	<1%	<1%

Table 2.

Demographic data and physical characteristics of the study group (n = 10).

Age (y)	25.0 ± 1.3
Alcohol per week (g/week)	35.9 ± 7.3
Body mass index (kg/m^2)	21.6 ± 0.6
Married (%)	2 (20%)
Children (n)	0.3 ± 0.2
Menstrual cycle length (days)	29.4 ± 0.8
Use of hormonal anticonceptives (%)	0
Tobacco users (%)	4 (40%)
Education, university/college (%)	8 (80%)
Employed/studying (%)	10 (100%)

Data are presented as mean ± standard error of the mean or n (%).

Table 3

Pharmacokinetic parameters of allopregnanolone after three injections, representing a cumulative dose of 0.09 mg/kg allopregnanolone, to 10 patients in the follicular phase of the menstrual cycle.

	Healthy women (n = 10) Mean ± SD
C_0 (nmol · l^{-1})	0.46 ± 0.2
C_5 (nmol · l^{-1})	20.9 ±9.7
C_{35} (nmol · l^{-1})	52.9 ± 26.2
C_{65} (nmol · l^{-1})	71.8 ± 29.0
$t_{½,1}$(min)	43.9 ± 7.3
$t_{½,z}$(min)	261 ± 100
AUC (nmol · l^{-1} · min)	8897 ± 1467
CL (ml · min^{-1} · kg^{-1})	32.6 ± 5.8
V_z (l · kg^{-1})	12.5 ± 6.3
V_{ss} (l · kg^{-1})	7.3 ± 2.5

C_0 endogenous serum concentration; C_5, C_{35}, C_{65} serum concentrations at 5 minutes after each allopregnanolone injection; $t_{½,1}$ distribution phase half-life; $t_{½,z}$ elimination phase half-life; AUC, area under the serum concentration/time curve; CL, clearance; V_z ,Volume of distribution in the elimination phase; V_{ss} ,Volume of distribution at steady state.

Figure 1

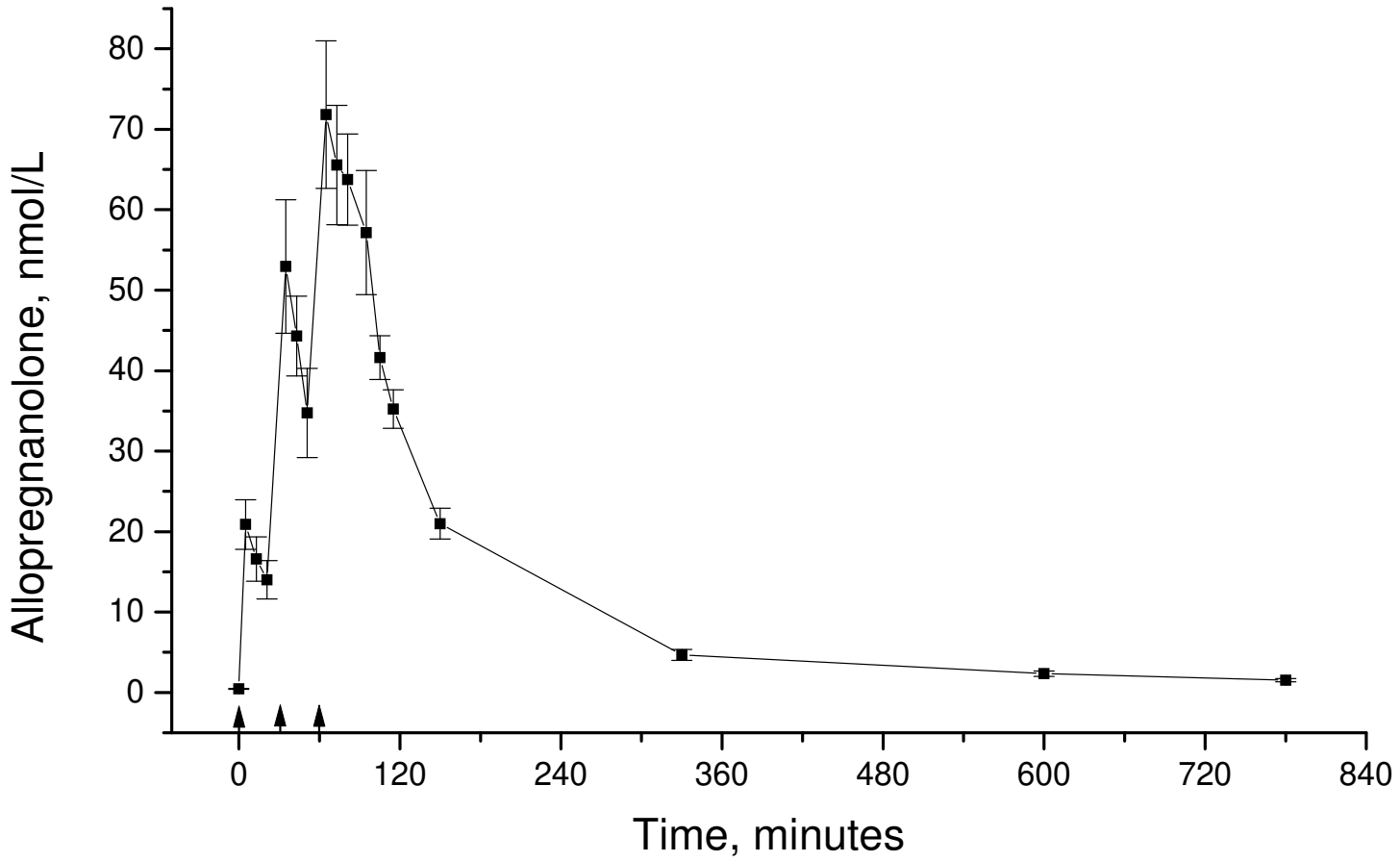

Allopregnanolone, nmol/L
0
10
20
30
40
50
60
70
80
0
120
240
360
480
600
720
840
Time, minutes

Figure 2

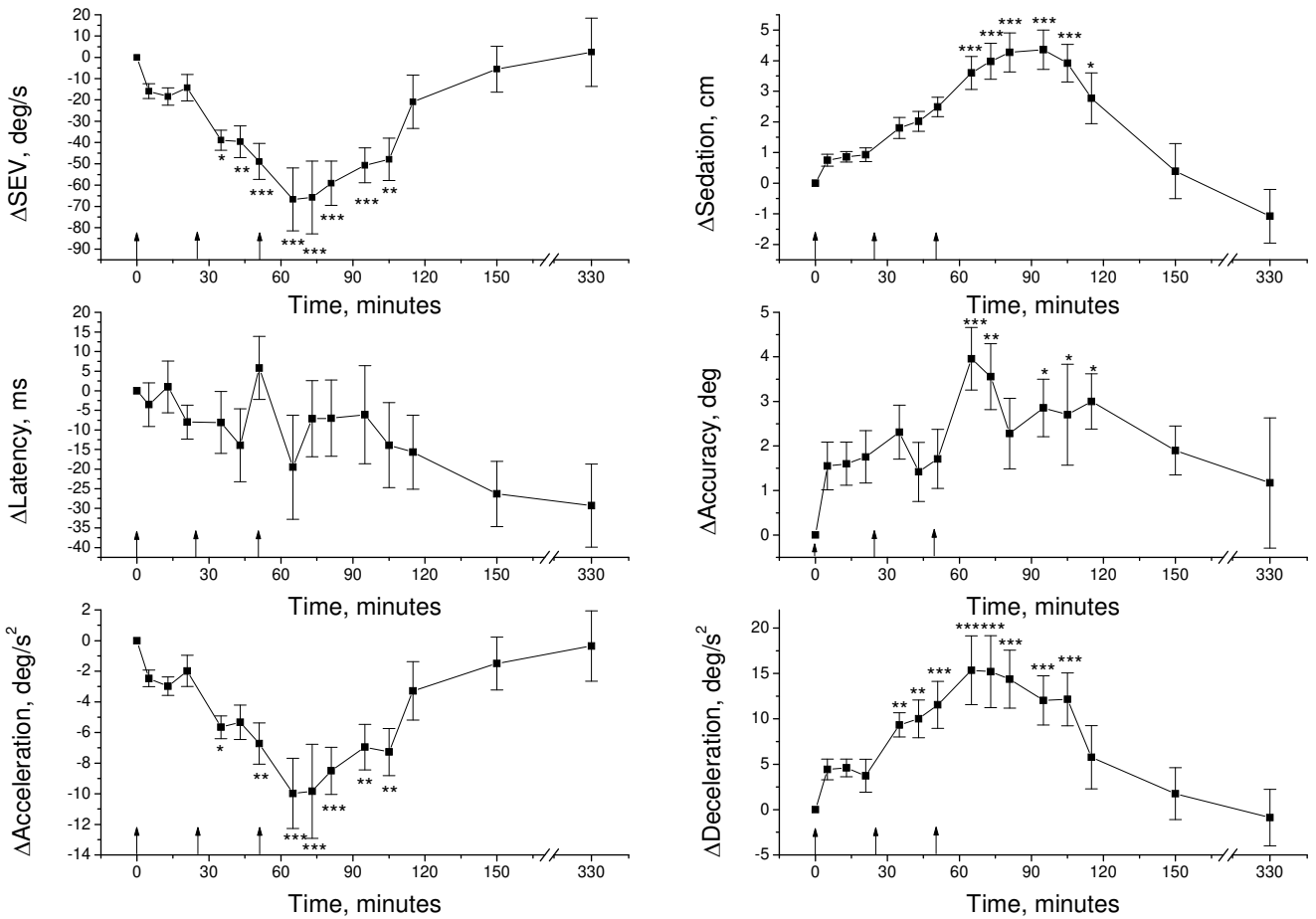
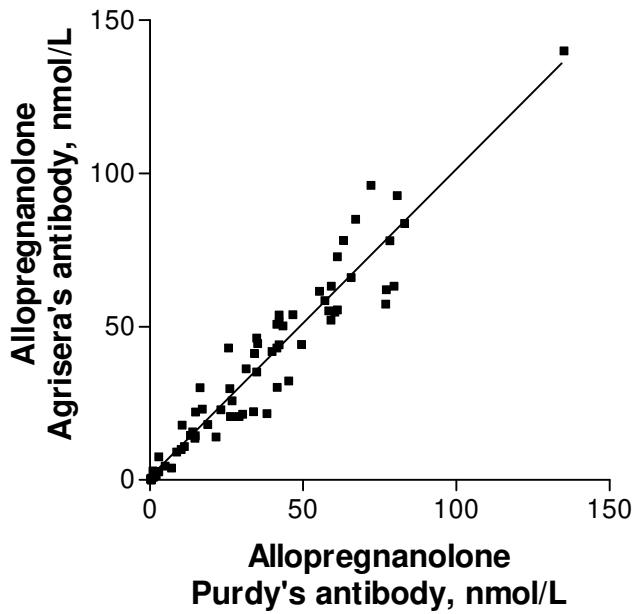

ΔSEV, deg/s
ΔSedation, cm
ΔLatency, ms
ΔAccuracy, deg
ΔAcceleration, deg/s2
ΔDeceleration, deg/s2
Time, minutes

Figure 3


Allopregnanolone
Agrisera's antibody, nmol/L
150
100
50
0
0
50
100
150
Allopregnanolone
Purdy's antibody, nmol/L

Legends

Figure 1

Serum concentration-time profile of allopregnanolone in the follicular phase of the menstrual cycle, values given as mean ± SEM., *n* = 10. Doses of allopregnanolone were 0.015, 0.03, and 0.045 mg/kg, and are indicated by *arrows*. The polyclonal rabbit antiserum was used for determination of allopregnanolone serum concentrations.

Figure 2

Allopregnanolone effect on saccade parameters and sedation scores were evaluated in 10 healthy women during the follicular phase of the menstrual cycle. Mean ± SEM of the change in saccadic eye velocity, saccade acceleration, saccade deceleration, saccade latency, saccade accuracy and visual analogue scores of sedation during the allopregnanolone challenge. During a saccade the eye velocity rises and falls smoothly, why maximum acceleration and maximum deceleration can be determined. Latency is the time lapse between the target movement to the onset of a saccade. Saccade accuracy is determined by comparing the actual eye position at the end of the saccade with the attempted target. * $p < 0.05$, Tukey Honestly Significance Test, ** $p < 0.01$, Tukey Honestly Significance Test, *** $p < 0.001$, Tukey Honestly Significance Test

Figure 3

Allopregnanolone, nmol/L, measured in serum samples from women during the follicular phase of menstrual cycle after iv injections of allopregnanolone 0.015, 0.03, and 0.045 mg/kg, respectively (n = 77). After celite chromatography separation, the serum samples were assayed with both a polyclonal rabbit antisera (Purdy et al. 1990) and the recently prepared

hen IgY antibody (Hen1102, Agrisera AB, Vännäs, Sweden). The correlation coefficient was $r = 0.962$; $p < 0.001$. The equation for the regression line was AlloAgrisera = 0.896 + 1.005 x AlloPurdy, $F(1,75)= 937$; $p<0.001$. The confidence limits of the line slope were 0.940 to 1.071 and the line broke the Y-axis at 0.896 nmol/L, 95% confidence interval -1.89 to 3.68, which is not different from zero.

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