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Isoallopregnanolone; an antagonist to the anaesthetic effect of allopregnanolone in male rats

Torbjörn Bäckström*, Göran Wahlström, Kerstin Wahlström, Di Zhu, Ming-De Wang

Umeå Neurosteroid Research Center, Department of Clinical Science, Obstetrics and Gynaecology, Umeå University, Umeå, Sweden
Umeå Neurosteroid Research Center, Department of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden

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Abstract

The interaction of isoallopregnanolone (3β-OH-5α-pregnan-20-one) on allopregnanolone (3α-OH-5α-pregnan-20-one) induced anaesthesia was studied in male rats using burst suppression of 1 s ("silent second") with an electroencephalographic-threshold method. The i.v. administration of isoallopregnanolone was varied in relation to induction of "silent second". Pre-treatment with isoallopregnanolone (12.5–50 mg/kg iv) 2 min prior to the threshold test gave an increase in the threshold dose of allopregnanolone (ANOVA $df(3;36)$, $F=13.61$, $P<0.001$), which was dose dependent ($r=0.73$, b [slope]=0.08, $df=38$, $P<0.001$). After isoallopregnanolone pre-treatment, but not in the controls, anaesthesia time was positively related to the dose of allopregnanolone ($r=0.52$, $b=1.72$, $df=28$, $P<0.01$). Anaesthesia times were not influenced by a corresponding administration of isoallopregnanolone immediately after induction of "silent second". When allopregnanolone and isoallopregnanolone were infused together at molar ratios of 1:1, 1:1.23, 1:1.43, a linear increase of the threshold doses of allopregnanolone was seen in relation to the dose of isoallopregnanolone ($r=0.86$, $b=0.40$, $df=8$, $P<0.01$). Thus isoallopregnanolone can antagonise the anaesthetic action of allopregnanolone.

Keywords: Allopregnanolone (3α-hydroxy-5α-pregnan-20-one); Isoallopregnanolone (3β-hydroxy-5α-pregnan-20-one); Neurosteroid antagonism; Anaesthesia threshold; Male; (Rat)

1. Introduction

It is well known that certain metabolites of progesterone, especially allopregnanolone (3α-OH-5α-pregnan-20-one), have pronounced inhibitory effects on brain excitability and can induce anaesthesia in both human and animals (Carl et al., 1990; Selye, 1942;). $GABA_A$-receptor-binding studies have shown that allopregnanolone enhances the γ-amino-butyric acid (GABA)-mediated Cl^- currents through action on a site (or sites) distinct from the GABA, benzodiazepine, barbiturate and picrotoxin binding sites (Gee et al., 1987; Majewska et al., 1986). This modulation site (or sites) shows a well-defined structure–activity relationship with a 3-α-hydroxy and a 20-ketone configuration in the pregnane molecule (Gee et al., 1987). Isoallopregnanolone (3β-OH-5α-pregnan-20-one), the 3-β isomer of allopregnanolone, has on the other hand in several reports showed no effect in vivo or in vitro (e.g. Bitran et al., 1991;Gee et al., 1987, Lundgren et al., 2003; Peters et al., 1988). However, with maximal GABA stimulation of recombinant receptors expressed in *Xenopus laevis* oocytes, isoallopregnanolone has shown an antagonistic effect against GABA (Wang et al., 2002). In recent in vitro studies, it has been shown that isoallopregnanolone can inhibit the effect of allopregnanolone on populations spikes in the CA1 hippocampal stratum pyramidale (Wang et al., 2000), non-competitively inhibit allopregnanolone's potentiating effect on GABA activated chloride current in recombinant expressed $GABA_A$ receptors (Wang et al., 2002) and antagonise the effect of allopregnanolone on Cl^- uptake in rat cortical microsacs

* Corresponding author. Umeå Neurosteriod Research Center, Department of Clinical Science, Obstetrics and Gynecology, Norrlands University Hospital, SE-901 85 Umeå, Sweden. Tel.: +46 90 77852144; fax: +46 90 776006.
E-mail address: Torbjorn.Backstrom@Obgyn.umu.se (T. Bäckström).

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(Lundgren et al., 2003). These results certainly merit further analyses of the effects of isoallopregnanolone in vivo.

In the present paper, we have studied the interaction between isoallopregnanolone and allopregnanolone on allopregnanolone-induced anaesthesia, which was monitored and confirmed with an in vivo electroencephalographic (EEG)-threshold technique. We have earlier assessed the anaesthetic potency of allopregnanolone with such an anaesthesia model (Norberg et al., 1987; Zhu et al., 2001). This technique is suitable for studying the interactions between depressant agents in the central nervous system (CNS). Changes in the threshold doses needed for induction with an active anaesthetic can easily be detected when combined with pre-treatment of defined doses of the interacting drug (Korkmaz and Wahlström, 1997). In the present paper, not only pre-treatment but also simultaneous and post-treatment administration of the assumed antagonist was investigated. After recording of threshold doses, anaesthesia times can easily be measured (Wahlström, 1966b). This variable was also used to study the effects of isoallopregnanolone.

2. Materials and methods

2.1. Animals

In the present study, a total of 98 male adult Sprague–Dawley rats (MOL: SPDR Möllegaard, Li, Skensved, Denmark) were used in three different experiments. 40 rats were used in Experiment 1 (Exp. 1). They were approximately 63 days old at the time of the experiment (body weight 333±3 g). 40 rats were used in Experiment 2 (Exp. 2). One rat was excluded due to technical problems. The rats in Exp. 2 were approximately 63 days old at the time of the experiment (body weight 327±2 g). 18 rats were used in Experiment 3 (Exp. 3). One rat was excluded due to technical problems. The rats in Exp. 3 were approximately 53 days old at the time of the experiment (body weight 242±2 g). All rats were housed three in each cage in a room with constant temperature of 24 °C and protected from all external light. A reversed day/night light schedule (light on between 19.00 and 07.00) was used in the rat room. The rats had access to water and standard rat food ad libitum. All parts of the study were approved by the Regional Ethics Committee for Animal Experiment in Umeå (Umeå djurförsöksetiska nämd).

2.2. Drugs

In Exps. 1 and 2, the stock solutions of allopregnanolone (Sigma Chemical, St. Louis, MO, USA) and isoallopregnanolone (Sigma Chemical, St. Louis, MO, USA) were made by dissolving allopregnanolone or isoallopregnanolone in 10% cyclodextrin (β-hydroxypropyl-cyclodextrin; Sigma Chemical, St. Louis, MO, USA) to a concentration of 4.0 mg/ml. The preparations were placed in the Bransonic 2210 ultrasonic bath for approximately 15 h and agitated occasionally. In Exp. 3, both the allopregnanolone and the isoallopregnanolone stock solutions were dissolved in 20% cyclodextrin to a concentration of 4.0 mg/ml. Allopregnanolone and isoallopregnanolone stock solutions were then mixed at ratios of 1:1, 1:1.23 and 1:1.43 to get the combined allopregnanolone/isoallopregnanolone solutions. The total steroid concentration in the combined solutions was kept at 4.0 mg/ml. In the controls of Exp. 3, the allopregnanolone stock solution was diluted with 20% β-cyclodextrin yielding a solution at a concentration of 2.0 mg/ml. The solution of all steroids in the vehicle was checked by visual inspection.

2.3. Electroencephalographic (EEG)-threshold method

The anaesthetic effect of allopregnanolone was in all experiments determined with an intravenous EEG-threshold method founded on a criterion of anaesthesia denoted the "silent second" (Korkmaz and Wahlström, 1997). The test solutions were administered as a continuous infusion via the tail vein. The syringe pump (Sage Instruments Model 355, Orion Research, USA) provided a constant speed of the infusion volume. The EEG was continuously recorded from two subcutaneous electrodes, with an ordinary EEG-recorder (Minograf EEG 10, Siemens Elma, Stockholm, Sweden). Stainless steel wire was used as electrodes, which was twisted and sewn into the head skin above the cortex in bifrontal position at least 24 h prior to the threshold test. The infusion of the solutions containing allopregnanolone was continued until the first burst suppression, which lasted 1 s or more, was recorded in the EEG. This threshold criterion is called "silent second " and indicates a stage of anaesthesia deeper than that needed to suppress the righting reflex (Wahlström, 1966a; Korkmaz and Wahlström, 1997). As a consequence anaesthesia times founded on the loss of righting reflex can always be measured after induction of the "silent second". The threshold dose of allopregnanolone was calculated by multiplying the time (min) to reach the "silent second" with the dose rate (mg/kg/min).

2.4. Design of the different experiments

In Exp. 1, an i.v. infusion of isoallopregnanolone was started 2 min before the start of the infusion with allopregnanolone. The rats were randomly allocated to four groups. Each separate group was either given the control solution (cyclodextrin) or different doses of isoallopregnanolone (12.5, 25 or 50 mg/kg) as an i.v. infusion during 1 min. Allopregnanolone was then infused with a dose rate of 4 mg/kg/min. Differences in weight of the rats were compensated by changes of the volume rate. The dose rate 4 mg/kg/min is the optimal dose rate for allopregnanolone as determined with the present solvent in an earlier experiment (Zhu et al., 2001). At the "silent second" criterion, the infusion was immediately stopped and the

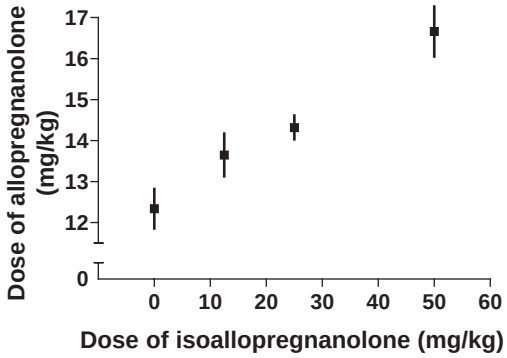

Fig. 1. Effect of pre-treatment with different doses of isoallopregnanolone on the threshold doses of allopregnanolone needed to induce a "silent second" in Exp. 1. Isoallopregnanolone was infused i.v. during 1 min starting 2 min prior to the infusion of allopregnanolone. There were 10 rats in each group total n=40.

anaesthesia time recorded using automatic recording beds described earlier (Wahlström, 1966b). Thus the anaesthesia time consisted of the time from the appearance of the first "silent second" in the EEG to the return of the righting reflex.

In Exp. 2, the rats were also randomly divided into four different groups. The control solution or three different doses of isoallopregnanolone were given in the same way as in Exp. 1 but the i.v. infusion started immediately after recording of the "silent second". Infusion of allopregnanolone was performed as in Exp. 1 and duration of anaesthesia was also measured in the same manner.

In Exp. 3, the rats were also divided into four randomly determined groups. Seven rats were given an i.v. infusion of the control allopregnanolone solution. Three, four and four rats were infused with combined allopregnanolone/isoallopregnanolone solutions at molar ratios of 1:1, 1:1.23, and 1:1.43, respectively. These solutions were infused to reach the criterion of the "silent second" in the EEG. Independent of the different mixtures used the dose rate of allopregnanolone was 2.65 mg/kg/min in all groups. Since the concentration of steroids in the mixture always was kept at 4.0 mg/ml, the dose rates of isoallopregnanolone differed depending on the concentrations in the mixtures. With a ratio of 1:1 the dose rate of isoallopregnanolone was 2.65 mg/kg/min, with a mixture of 1:1.23 the dose rate of isoallopregnanolone was 3.26 mg/kg/min and with a mixture of 1:1.43 the dose rate of isoallopregnanolone was 3.78 mg/kg/min. To keep a constant dose rate of allopregnanolone, the volume rate was used to compensate for differences in concentrations of allopregnanolone and differences in weight of the rats according to the following formula: Volume rate=Dose rate*Weight/Concentration (ml/min=(mg/kg/min)*kg/(mg/ml)).

2.5. Statistical analysis

The differences of the threshold doses for allopregnanolone among doses of isoallopregnanolone groups were assessed by analysis of variance (ANOVA) followed ad hoc by least significant difference test. Differences in anaesthesia times were analysed in the same way. Linear parametric regression coefficients and corresponding correlation coefficients were used to study dose/effect relationships. All statistical calculations were performed using StatView statistical software. Value of P<0.05 in the two-tailed test was taken to represent significant differences. NS indicated non-significant differences. All results are presented as means with one standard error of mean (S.E.M); *df* indicates degrees of freedom.

3. Results

In Exp. 1, the rats were pre-treated with different i.v. doses of isoallopregnanolone administered 2 min before starting the threshold determination. The result is shown in Fig. 1. It is evident that in the presence of isoallopregnanolone, there was a clear, isoallopregnanolone-dependent increase in the doses of allopregnanolone needed to reach the "silent second" (ANOVA *df*(3;36), F=13.61, P<0.001). There was also a corresponding significant regression between the doses of isoallopregnanolone and the dose of allopregnanolone needed to induce the "silent second" (r=0.73, b=0.08, *df*=38, P<0.001). The ensuing anaesthesia times are given in Fig. 2. In the controls there was no

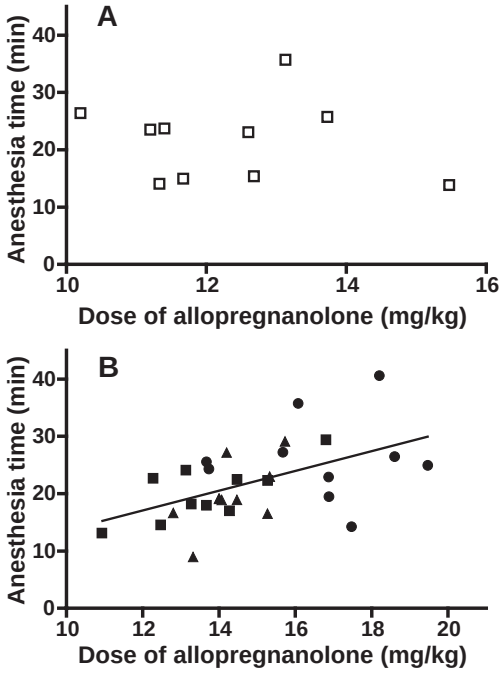

Fig. 2. Relation between the dose of allopregnanolone needed to induce the "silent second" and the duration of the ensuing anaesthesia times in Exp. 1. Panel A shows the result in the control rats (open squares). Panel B shows the result in the isoallopregnanolone pre-treated rats. The pre-treatment in these groups was: 12.5 mg/kg (filled squares), 25 mg/kg (filled triangles) and 50 mg/kg (filled circles). The equation of the regression line is y=2.06+1.72x; r=0.52, P<0.01.

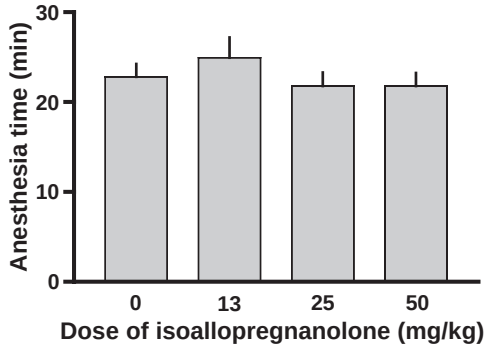

Fig. 3. Effect of different doses of isoallopregnanolone on the anaesthesia times in Exp. 2 (mean±S.E.M.). Isoallopregnanolone was administered i.v. immediately after induction of the "silent second" with allopregnanolone. There were 10 rats in each group except in the group given 25 mg/kg of isoallopregnanolone where there were 9 rats.

relation between the doses of allopregnanolone needed to induce the "silent second" and the anaesthesia time (Fig. 2A). However, in the groups of animals pre-treated with isoallopregnanolone, there was a significant positive relation between the dose of allopregnanolone and the duration of the anaesthesia (r=0.52, b=1.72, df=28, P<0.01, Fig. 2B).

To evaluate the direct effect of isoallopregnanolone on the duration of anaesthesia, isoallopregnanolone was in Exp. 2 given in different doses i.v. immediately after induction of the "silent second" with allopregnanolone. As expected there was no difference in dose needed to induce the "silent second" between the different groups (11.1±0.2 mg/kg; n=39; ANOVA df(3;35), F=0.60, NS). Fig. 3 shows that the administration of different doses of isoallopregnanolone had no direct effect on the anaesthesia times (ANOVA df(3;35), F=0.76, NS).

In Exp. 3, isoallopregnanolone was infused together with allopregnanolone until induction of the "silent second". Control solution and three different mixtures of allopregnanolone and isoallopregnanolone were given and the result is shown in Fig. 4. Compared with the controls and depending on the admixture of isoallopregnanolone, increasing doses of allopregnanolone were needed to obtain the "silent second" (ANOVA df(3;13), F=4.48, P<0.05). There was also in the admixture doses a positive significant correlation between the dose of isoallopregnanolone and the dose of allopregnanolone needed to induce the "silent second" (r=0.86, b=0.40, df=8, P<0.01). The regression line founded on the admixture data cuts the level of the dose of allopregnanolone needed in the controls (12.8±0.3 mg/kg) at 13.2 mg/kg of isoallopregnanolone.

4. Discussion

The main result of this report is that isoallopregnanolone inhibits the anaesthetic effect of allopregnanolone if given intravenously to male rats prior to or simultaneously with allopregnanolone. However, isoallopregnanolone does not shorten the anaesthesia time if given after the anaesthesia is induced. This antagonism of isoallopregnanolone against allopregnanolone in Exp. 1 was shown by a dose-dependent increased in threshold dose of allopregnanolone needed to induce the anaesthetic criterion. A similar dose-dependent increase was also seen in Exp. 3 where isoallopregnanolone was infused at different dose rates together with allopregnanolone. This is to our knowledge the first time that isoallopregnanolone has been shown to dose dependently antagonise the anaesthetic action of allopregnanolone in an in vivo model. The site of interaction between isoallopregnanolone and allopregnanolone is not investigated in the present paper and can be both at a pharmacokinetic and pharmacodynamic level. A pharmacodynamic interaction would possibly be at a receptor directly involved in the induction of anaesthesia and where allopregnanolone acts. A strong candidate is the $GABA_A$ receptor since it is involved in anaesthesia (Mehta and Ticku, 1999) and several studies show that allopregnanolone interacts with the $GABA_A$ receptor and has no effects on other receptors (Majewska et al., 1986; Gee et al., 1987; Peters et al., 1988; Turner et al., 1989). In favour of a pharmacodynamic hypothesis is that in vitro studies show antagonism by isoallopregnanolone on allopregnanolone-enhanced GABA-mediated increase in chloride current where the pharmacokinetic interaction is minimized by direct applications of the substances to the cell preparations (Wang et al., 2002, Lundgren et al., 2003). In addition Prince and Simmonds (1993) reported that isoallopregnanolone increased the EC50 values for allopregnanolone to potentiate [3H]flunitrazepam binding, while the maximal potentiation was not changed. In hippocampus slices, direct tissue application isoallopregnanolone dose dependently antagonise the allopregnanolone inhibition of population spikes (Wang et al., 2000). Thus the results in the present experiments, showing that isoallopregnanolone can antagonise the anaesthetic effect of allopregnanolone, suggest a pharmacodynamic interaction between the two steroids. The site for this

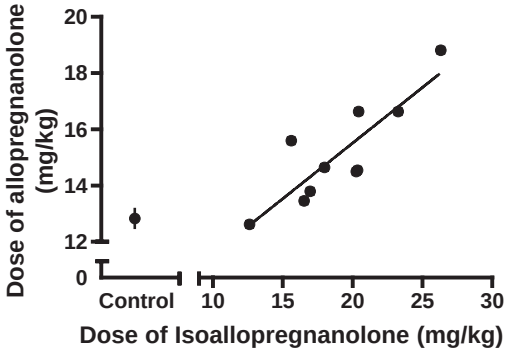

Fig. 4. Threshold dose of allopregnanolone needed to induce the "silent second" when administered together with isoallopregnanolone in Exp. 3. The controls were co-administered with allopregnanolone+solvent instead of the solution with isoallopregnanolone. The regression line is calculated only on the results of admixture doses not including the control. The equation of regression line is y=−19.2+0.40x; r=0.86, P<0.01.

interaction is probably located on the GABA ionophore. However, the indication of a pharmacodynamic interaction does not rule out the possibilities of a concomitant pharmacokinetic interaction. This issue could probably be resolved if we could measure the concentrations of isoallopregnanolone and allopregnanolone together in different brain areas after induction and at the end of anaesthesia.

Pharmacokinetic factors could be involved in the different potency of isoallopregnanolone to antagonise the dose of allopregnanolone recorded in Exp. 1 and Exp. 3. In Exp. 1, pre-treatment with 25 mg/kg of isoallopregnanolone increased the threshold dose of allopregnanolone with approximately 17% of the value recorded in the controls (Fig. 1). The corresponding increase in threshold dose when 25 mg/kg of isoallopregnanolone was administered together with allopregnanolone (Exp. 3) was approximately 36%. This increase in potency of isoallopregnanolone when given together with allopregnanolone indicates a time-dependent interaction between the two steroids at a receptor involved in the anaesthesia probably the $GABA_A$ receptor. One explanation to the difference in potency could be that affinity of isoallopregnanolone is low and that the elimination of isoallopregnanolone from the receptor-binding site is very rapid. This means that already 1 min after the infusion some isoallopregnanolone could have dissociated. Due to the increased potency of isoallopregnanolone in Exp. 3 and the chemical similarities between the steroids, a direct reversible competition at such a specific receptor site could be expected. However, earlier in vitro studies have rather pointed to a non-competitive antagonism between isoallopregnanolone and allopregnanolone (Wang et al., 2002, Lundgren et al., 2003).

The lack of prolongation of the anaesthesia time in Exp. 2, when isoallopregnanolone was administered after induction of anaesthesia with allopregnanolone, needs some further consideration. First we have to realize the limitations when working with a threshold technique. If the criterion used for induction of anaesthesia (the "silent second") and the criterion used to record the end of the effect (return of righting reflex) are related, one would not expect the duration of the anaesthesia time to change if the threshold dosage is changed. This occurs when both the threshold dosage and anaesthesia time are dependent on the sensitivity of the individual rat to allopregnanolone. This has earlier clearly been shown for hexobarbital as an induction agent both in normal and tolerant rats (Wahlström, 1966a, 1974). A prerequisite is that there is no change in the pharmacokinetic situation neither with regard to metabolism nor with regard to redistribution of the tested drug. In the present experiments, both pharmacokinetic prerequisites should also apply to isoallopregnanolone. However, in Exp. 1 the threshold dose for allopregnanolone increased with increasing dose of isoallopregnanolone (Fig. 1) and the duration of the anaesthesia time also increased after this pre-treatment (Fig. 2). These results are clearly different from the expected. A puzzling result is that no corresponding increase in anesthesia time was recorded in Exp. 2 (Fig. 3) where the treatment with isoallopregnanolone was given after the "silent second" had been recorded. We can only speculate on the reasons, as this question was not further investigated in the present paper. One explanation could be that some of isoallopregnanolone was metabolised to allopregnanolone (Barnea et al., 1990). An inter-conversion between allopregnanolone and isoallopregnanolone can occur in the brain tissue. (Vallée et al., 2000; Mellon and Griffin, 2002) and that the antagonistic effect of isoallopregnanolone is counteracted by a metabolism to allopregnanolone and that abolished the antagonistic effect. Another alternative could be a rapid redistribution of isoallopregnanolone, which was not recorded in Exp. 1. A third alternative is that there exists a specific sensitivity to the effect of isoallopregnanolone at the induction of anesthesia, but a lack of effect at the return of the righting reflex. In this situation isoallopregnanolone could not influence the anesthesia times and the return of the righting reflex was only accomplished by the disappearance of allopregnanolone.

Isoallopregnanolone alone has been tested in various in vivo behavioral experiments to investigate its own action in the central nervous system. In old reports a moderately potent anesthetic effect of isoallopregnanolone was shown when given in high dosages intravenously in a suspension to mice (Figdor et al., 1957). In another study (Atkinson et al., 1965), no anesthetic activity but occasional convulsions was found after intravenous isoallopregnanolone in high doses. Gyermek et al. (1968) studied the structure–activity relationship of steroid hypnotic agents. They observed a loss of hypnotic potency when the steric position of the 3α-OH group was altered and isoallopregnanolone proved to be ineffective up to 200 mg/kg when given intraperitoneally in mice. When interpreting the old literature, it has to be kept in mind that the solvents used for the steroids in these studies often were toxic and have effects in the central nervous system by themselves which in some cases also could interact with the steroid (Gyermek et al., 1968; Österlind et al., 1979). Furthermore the purity of the steroid composition in the solution could sometimes be questioned.

In modern literature, there are now a large number of in vivo studies in rats and mice showing effects by allopregnanolone on functional and behavioral measures but where isoallopregnanolone by it self has showed no effect, e.g. anxiolytic effects (Bitran et al., 1991; Carboni et al., 1996; Rodgers and Johnson, 1998; Wieland et al., 1991), antiepileptic effects (Kokate et al., 1994), food intake and feeding behaviour (Chen et al., 1996) or stimulated gastric acid secretion (Watanabe et al., 2000). In all these situations, isoallopregnanolone has been tested for its own effect and not as a potential antagonist to the allopregnanolone effect.

Isoallopregnanolone alone has also been tested in pharmacological and neurophysiological in vitro experiments using several different techniques and has not shown

any change in GABA mediated effects. (Gee et al., 1987; Lan et al., 1991; Lundgren et al., 2003; Kokate et al., 1994; Peters et al., 1988; Prince and Simmonds, 1993; Smith et al., 1987). The conclusion from the modern literature is that isoallopregnanolone alone has no allopregnanolone-like effect on the $GABA_A$ receptor.

The present results could be of clinical interest in the future as it opens up a possibility to use isoallopregnanolone to treat symptoms and effects induced by allopregnanolone. Allopregnanolone has not been used to induce anesthesia in humans but an isomer pregnanolone has. Bolus injections of 0.4–0.6 mg/kg pregnanolone induce a short and superficial anesthesia in humans. The serum concentrations were in that situation between 0.5 and 1.7 μM (Carl et al., 1990). The serum concentrations of allopregnanolone in rats at a much deeper anesthesia, that is at ”silent second”, are depending on the dose rate but at optimal dose rate a dose of 16 mg/kg gives a concentration around 50 μM. (Zhu et al., 2001). Allopregnanolone is produced during the luteal phase of the menstrual cycle in women (Wang et al., 1996). Allopregnanolone increases also in the rat brain at stress (Purdy et al., 1991) and some of the negative effects of acute stress might be related to the effects of allopregnanolone. Negative symptoms induced by allopregnanolone are CNS depression (Norberg et al., 1987; Zhu et al., 2001), memory impairment (Mayo et al., 1993; Johansson et al., 2002), induction of increased frequency of petite mal seizures (Bäckström et al., 1983, Banerjee and Snead, 1998; Grünewald et al., 1992) and increased food consumption with possible obesity as result (Chen et al., 1996). Increased number of epileptic seizures and migraine attacks are noted at menstruation in women with partial epilepsy with migraine indicating a withdrawal effect (Bäckström, 1976; MacGregor, 1996). A decreased function of the $GABA_A$ system due to tolerance development is seen at prolonged and continuous presence of allopregnanolone (Yu and Ticku, 1995; Marshall et al., 1997, Zhu et al., 2004). This may explain why women with premenstrual dysphoric disorder during the luteal phase are less sensitive to the sedative effect of benzodiazepines, pregnanolone and alcohol (Sundström et al., 1997, 1998; Nyberg et al., 2004). All these negative effects by allopregnanolone might be reversed after treatment with an antagonist. The in vivo results presented here open up a new concept of treatment of symptoms caused by the naturally occurring $GABA_A$-receptor modulating substance allopregnanolone.

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References

Atkinson, R.M., Davis, B., Pratt, M.A., Sharpe, H.M., Tomich, E.G., 1965. Action of some steroids on the central nervous system of the mouse: II. Pharmacology. J. Med. Chem. 8, 426–432.

Bäckström, T., 1976. Epileptic seizures in women related to plasma estrogen and progesterone during the menstrual cycle. Acta Neurol. Scand. 54, 321–347.

Bäckström, T., Baird, D.T., Bancroft, J., Bixo, M., Hammarbäck, S., Sanders, D., Smith, S., Zetterlund, B., 1983. Endocrinological aspects on cyclical mood changes or the premenstrual syndrome. J. Psychosom. Obstet. Gynaecol. 2, 8–20.

Banerjee, P.K., Snead III, O.C., 1998. Neuroactive steroids exacerbate gamma-hydroxybutyric acid-induced absence seizures in rats. Eur. J. Pharmacol. 359, 41–48.

Barnea, A., Hajibeigi, A., Trant, J.M., Mason, J.I., 1990. Expression of steroid metabolising enzymes by aggregating fetal brain cells in culture: a model for developmental regulation of the progesterone 5 alpha-reductase pathway. Endocrinology 127, 500–502.

Bitran, D., Hilvers, R.J., Kellogg, C.K., 1991. Anxiolytic effects of 3 alpha-hydroxy-5 alpha[beta]-pregnan-20-one: endogenous metabolites of progesterone that are active at the GABAA receptor. Brain Res. 561, 157–161.

Carboni, E., Wieland, S., Lan, N.C., Gee, K.W., 1996. Anxiolytic properties of endogenously occurring pregnanediols in two rodent models of anxiety. Psychopharmacology 126, 173–178.

Carl, P., Högskilde, S., Nielsen, J.W., Sörensen, M.B., Lindholm, M., Karlen, B., Bäckström, T., 1990. Pregnanolone emulsion. A preliminary pharmacokinetic and pharmacodynamic study of a new intravenous anaesthetic agent. Anaesthesia 45, 189–197.

Chen, S.H., Rodriguez, L., Davies, M.F., Loew, G.H., 1996. The hyperphagic effect of 3-alfa-hydroxylated pregnane steroids in male rats. Pharmacol. Biochem. Behav. 53, 772–782.

Figdor, S.K., Kodet, M.J., Bloom, B.M., Agnello, E.J., P'an, S.Y., Laubach, G.D., 1957. Central activity and structure in a series of water-soluble steroids. J. Pharmacol. Exp. Ther. 119, 299–309.

Gee, K.W., Chang, W.-C., Brinton, R.E., McEwen, B.S., 1987. GABA-dependent modulation of the Cl^- ionophore by steroids in rat brain. Eur. J. Pharmacol. 136, 419–423.

Grünewald, R.A., Aliberti, V., Panayiotopoulos, C.P., 1992. Exacerbation of typical absence seizures by progesterone. Seizure 1, 137–138.

Gyermek, L., Iriarte, J., Crabbé, P., 1968. Steroids. CCCX. Structure–activity relationship of some steroidal hypnotic agents. J. Med. Chem. 11, 117–125.

Johansson, I.M., Birzniece, V., Lindblad, C., Olsson, T., Bäckstrom, T., 2002. Allopregnanolone inhibits learning in the Morris water maze. Brain Res. 934, 125–131.

Kokate, T.G., Svensson, B.E., Rogawski, M.A., 1994. Anticonvulsant activity of neurosteroids: correlation with gamma-aminobutyric acid-evoked chloride current potentiation. J. Pharmacol. Exp. Ther. 270, 1223–1229.

Korkmaz, S., Wahlström, G., 1997. The EEG burst suppression threshold test for the determination of CNS sensitivity to intravenous anaesthetics in rats. Brain Res. Protoc. 1, 378–384.

Lan, N.C., Gee, K.W., Bolger, M.B., Chen, J.S., 1991. Differential responses of expressed recombinant human gamma-aminobutyric acidA receptors to neurosteroids. J. Neurochem. 57, 1818–1821.

Lundgren, P., Strömberg, J., Bäckström, T., Wang, M., 2003. Allopregnanolone-stimulated GABA-mediated chloride ion flux is inhibited by 3β-OH-5α-pregnan-20-one (isoallopregnanolone). Brain Res. 982, 45–53.

MacGregor, E.A., 1996. “Menstrual” migraine: towards a definition. Cephalalgia 16, 11–21.

Majewska, M.D., Harrison, N.L., Schwartz, R.D., Barker, J.L., Paul, S.M., 1986. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232, 1004–1007.

Marshall, F.H., Stratton, S.C., Mullings, J., Ford, E., Worton, S.P., Oakley, N.R., Hagan, R.M., 1997. Development of tolerance in mice to the

sedative effects of the neuroactive steroid minaxolone following chronic exposure. Pharmacol. Biochem. Behav. 58, 1–8.

Mayo, W., Dellu, F., Robel, P., Cherkaoui, J., Le Moal, M., Baulieu, E.-E., Simon, H., 1993. Infusion of neurosteroids into the nucleus basalis magnocellularis affects cognitive processes in the rat. Brain Res. 607, 324–328.

Mehta, A.K., Ticku, M.K., 1999. An update on $GABA_A$ receptors. Brain Res. Rev. 29, 196–217.

Mellon, S.H., Griffin, L.D., 2002. Neurosteroids: biochemistry and clinical significance. Trends Endocrinol. Metab. 13, 35–43.

Norberg, L., Wahlström, G., Bäckström, T., 1987. The anaesthetic potency of 3α-OH-5α-pregnan-20-one and 3α-OH-5β-pregnan-20-one determined with an intravenous EEG-threshold method in male rats. Pharmacol. Toxicol. 61, 42–47.

Nyberg, S., Wahlström, G., Bäckström, T., Sundström-Poromaa, I., 2004. Altered sensitivity to alcohol among patients with premenstrual dysphoric disorder. Psychoneuroendocrinology 29, 767–777.

Österlind, A., Åkesson, Å., Wahlström, G., 1979. Interactions between 1.2-propanediol (propylene glycol) and hexobarbital. Acta Pharmacol. Toxicol. 45, 245–248.

Peters, J.A., Kirkness, E.F., Callachan, H., Lambert, J.J., Turner, A.J., 1988. Modulation of the GABAA receptor by depressant barbiturates and pregnane steroids. Br. J. Pharmacol. 94, 1257–1269.

Prince, R.J., Simmonds, M.A., 1993. Differential antagonism by epipregnanolone of alphaxalone and pregnanolone potentiation of [3H]flunitrazepam binding suggests more than one class of binding site for steroids at GABAA receptors. Neuropharmacology 32, 59–63.

Purdy, R.H., Morrow, A.L., Moore Jr., P.H., Paul, S.M., 1991. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. Proc. Natl. Acad. Sci. 88, 4553–4557.

Rodgers, R.J., Johnson, N.J., 1998. Behaviorally selective effects of neuroactive steroids on plus-maze anxiety in mice. Pharmacol. Biochem. Behav. 59, 221–232.

Selye, H., 1942. Correlations between the chemical structure and the pharmacological actions of the steroids. Endocrinology 30, 437–453.

Smith, S.S., Waterhouse, B.D., Woodward, D.J., 1987. Locally applied progesterone metabolites alter neuronal responsiveness in the cerebellum. Brain Res. Bull. 18, 739–747.

Sundström, I., Ashbrook, D., Bäckström, T., 1997. Reduced benzodiazepine sensitivity in patients with premenstrual syndrome: a pilot study. Psychoneuroendocrinology 22, 25–38.

Sundström, I., Andersson, A., Nyberg, S., Ashbrook, D., Purdy, R.H., Bäckström, T., 1998. Patients with premenstrual syndrome have a different sensitivity to a neuroactive steroid during the menstrual cycle compared to control subjects. Neuroendocrinology 67, 126–138.

Turner, D.M., Ransom, R.W., Yang, J.S.-J., Olsen, R.W., 1989. Steroid anesthetics and naturally occurring analogs modulate the gamma-aminobutyric acid receptor complex at a site distinct from barbiturates. J. Pharmacol. Exp. Ther. 248, 960–966.

Vallée, M., Rivera, J.D., Koob, G.F., Purdy, R.H., Fitzgerald, R.L., 2000. Qantification of neurosteroids in rat plasma and brain following swim stress and allopregnanolone administration using negative chemical ionization gas chromatography/mass spectrometry. Anal. Chem. 287, 153–166.

Wahlström, G., 1966a. Estimation of brain sensitivity to hexobarbitone (Enhexymal NFN) in rats by an EEG threshold. Acta Pharmacol. Toxicol. 24, 404–418.

Wahlström, G., 1966b. Hexobarbitone sleeping time in rats following doses with similar EEG changes. Acta Pharmacol. Toxicol. 24, 419–434.

Wahlström, G., 1974. Withdrawal in the rat after long-term foreced oral barbital administration. Acta Pharmacol. Toxicol. 35, 131–144.

Wang, M.D., Seippel, L., Purdy, R.H., Bäckström, T., 1996. Relationship between symptom severity and steroid variation in women with premenstrual syndrome: study on serum pregnenolone, pregnenolone sulfate, 5 alpha-pregnane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one. J. Clin. Endocrinol. Metab. 81, 1076–1082.

Wang, M.D., Bäckström, T., Landgren, S., 2000. The inhibitory effects of allopregnanolone and pregnanolone on the population spike, evoked in the rat hippocampal CA1 stratum pyramidale in vitro, can be blocked selectively by epiallopregnanolone. Acta Physiol. Scand. 169, 333–341.

Wang, M., He, Y., Eisenman, L.N., Fields, C., Zeng, C.-M., Mathews, J., Benz, A., Fu, T., Zorumski, E., Steinbach, J.H., Covey, D.F., Zorumski, C.F., Mennerick, S., 2002. 3beta-hydroxypregnane steroids are pregnenolone sulfate-like GABAA receptor antagonists. J. Neurosci. 22, 3366–3375.

Watanabe, K., Nagakura, Y., Hiura, N., Tsuchiya, S., Horie, S., 2000. Stimulation of gastric acid secretion by progesterone metabolites as neuroactive steroids in anesthetized rats. J. Physiol. (Paris) 94, 111–116.

Wieland, S., Lan, N.C., Mirasedeghi, S., Gee, K.W., 1991. Anxiolytic activity of the progesterone metabolite 5α-pregnan-3α-ol-20-one. Brain Res. 565, 263–268.

Yu, R., Ticku, M.K., 1995. Chronic neurosteroid treatment decreases the efficacy of benzodiazepine ligands and neurosteroids at the gamma-aminobutyric acidA receptor complex in mammalian cortical neurons. J. Pharmacol. Exp. Ther. 275, 784–789.

Zhu, D., Wang, M.D., Bäckström, T., Wahlström, G., 2001. Evaluation and comparison of the pharmacokinetic and pharmacodynamic properties of allopregnanolone and pregnanolone at induction of anaesthesia in the male rat. Br. J. Anaesth. 86, 403–412.

Zhu, D., Birzniece, V., Bäckström, T., Wahlström, G., 2004. Dynamic aspects on acute tolerance to allopregnanolone evaluated using an anaesthesia threshold in male rats. Br. J. Anaesth. 93, 560–567.