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INVITED REVIEW

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Positive $GABA_A$ receptor modulating steroids and their antagonists: Implications for clinical treatments

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Abstract

GABA is the main inhibitory neurotransmitter in the brain and GABAergic transmission has been shown to be of importance for regulation of mood, memory and food intake. The progesterone metabolite allopregnanolone (Allo) is a positive $GABA_A$ receptor modulating steroid with potent effects. In humans, disorders such as premenstrual dysphoric disorder (PMDD), hepatic encephalopathy and polycystic ovarian syndrome are associated with elevated Allo levels and increased negative mood, disturbed memory and increased food intake in some individuals. This is surprising because Allo shares many properties with benzodiazepines and is mainly considered to be anxiolytic and anti-depressant. However, it is well established that, in certain individuals, $GABA_A$ receptor activating compounds could have paradoxical effects and thus be anxiogenic in low physiological plasma concentrations but anxiolytic at high levels. We have demonstrated that isoallopregnanolone (Isoallo), the 3β-OH sibling of Allo, functions as a $GABA_A$ receptor modulating steroid antagonist (GAMSA) but without any effects of its own on $GABA_A$ receptors. The antagonistic effect is noted in most $GABA_A$ subtypes investigated in vitro to date. In vivo, Isoallo can inhibit Allo-induced anaesthesia in rats, as well as sedation or saccadic eye velocity in humans. Isoallo treatment has been studied in women with PMDD. In a first phase II study, Isoallo (Sepranolone; Asarina Pharma) injections significantly ameliorated negative mood in women with PMDD compared with placebo. Several GAMSAs for oral administration have also been developed. The GAMSA, UC1011, can inhibit Allo induced memory disturbances in rats and an oral GAMSA, GR3027, has been shown to restore learning and motor coordination in rats with hepatic encephalopathy. In humans, vigilance, cognition and pathological electroencephalogram were improved in patients with hepatic encephalopathy on treatment with GR3027. In conclusion GAMSAs are a new possible treatment for disorders and symptoms caused by hyperactivity in the $GABA_A$ system.

KEYWORDS

allopregnanolone, $GABA_A$ receptor modulating steroid antagonists, isoallopregnanolone

1 | INTRODUCTION

It is common knowledge that altered GABA-ergic function is associated with neurological and psychiatric disorders. Neuroactive steroids, mainly metabolites of stress and sex steroids (eg, deoxycorticosterone and progesterone) are known to be potent positive modulators of the $GABA_A$ receptor. However, the existence of negative $GABA_A$ receptor modulators and antagonists to positive steroid $GABA_A$ receptor modulators is perhaps less well known. Extensive research has shown that positive $GABA_A$ receptor modulators such as the neuroactive steroid allopregnanolone (Allo) have pronounced effects in the brain. Some of these effects are beneficial, and allopregnanolone is thus promoted as a therapy for neuropsychiatric disorders,[1] although some behavioural and neurological effects are aversive and disliked by the patients. Below, we discuss the clinical disorders and symptoms that are related to increased neuroactive steroid concentrations and/or increased sensitivity to neuroactive steroids. We will also discuss some experimental animal studies and clinical human studies of the effects of isoallopregnanolone (Isoallo) and an oral compound (GR3027) that are $GABA_A$ receptor modulating steroid antagonists (GAMSAs), opening up the possibility that the negative effects of $GABA_A$ receptor modulating steroids can be counteracted.

2 | CLINICAL SITUATIONS WHERE THERE IS A TEMPORAL RELATIONSHIP BETWEEN THE PRESENCE OF NEUROACTIVE STEROIDS AND SYMPTOMS

There are several situations where production of neuroactive steroids and plasma concentrations are naturally fluctuating and through which it is possible to detect relationships between symptoms and variations in steroid blood concentrations. For example, both Allo and Isoallo are produced by the corpus luteum of the ovary or secreted from the adrenal cortex during stress and Allo is also synthesised directly in the brain.[2,3] The existence of such relationships is discussed below, although there are limitations to the interpretation since neuroactive steroids produced within the brain can reach effective levels locally without any corresponding variation in peripheral blood. However, we know that an increase in blood concentrations is mirrored in the brain and influences brain activity.[4] Examples of situations with fluctuating levels of neuroactive steroids are during the menstrual cycle, pregnancy and the post-partum period in women, as well as during acute and chronic stress. There are also specific disorders associated with an increased brain concentration of neuroactive steroids (eg, hepatic encephalopathy).[5]

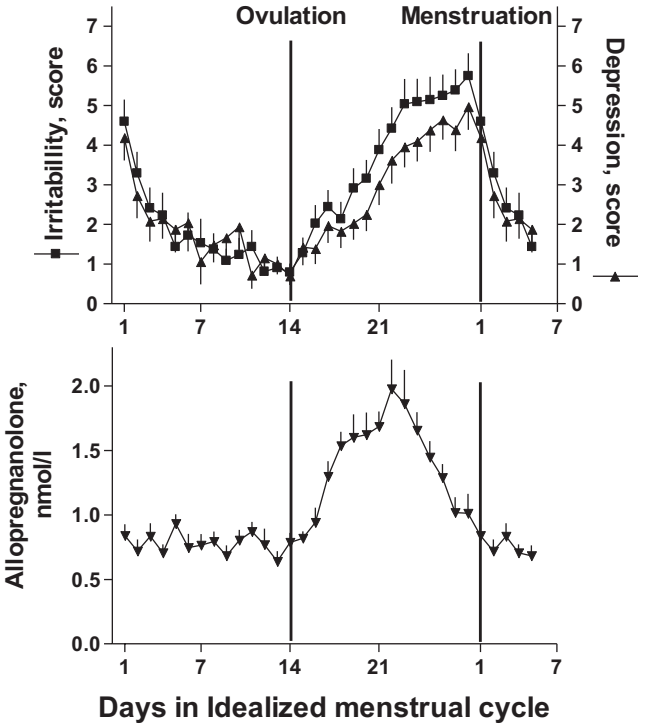

FIGURE 1 Symptom-allopregnanolone relationship in premenstrual dysphoric disorder (PMDD) patients during the menstrual cycle. Irritability score curve correlates best with allopregnanolone serum concentration. N = 38 cycles in 19 patients. Reprinted with permission from Bäckström et al[103]

3 | EXAMPLES OF DISORDERS AND SYMPTOMS RELATED TO VARIATIONS IN NEUROACTIVE STEROID LEVELS

3.1 | Mood disorders

In premenstrual dysphoric disorder/premenstrual syndrome (PMDD/PMS), the symptoms arrive close to the allopregnanolone rise after ovulation at the beginning of the luteal phase. Symptom severity continues to rise until the end of the menstrual cycle and the start of the full flow of menstrual bleeding (Figure 1).[6] In the beginning of the next menstrual cycle, the symptoms decline rapidly and usually disappear within 3-4 days of the onset of menstrual bleeding. During the postmenstrual phase, there is a period of well-being, closely related to oestrogen production, ending with the oestradiol peak[6] (Figure 1). This pattern suggests that a symptom-provoking factor is indeed produced by the corpus luteum of the ovary. This is further supported by the finding that, in anovulatory cycles, either spontaneous or induced, Allo is not produced and no symptom cyclicity occurs.[7,8] The menstrual cycle is thus a period of naturally fluctuating production of neuroactive steroids, with very low Allo levels during the follicular phase and between 10-20

times higher levels during the luteal phase. Plasma concentrations have been measured in several mood disorders. PMDD/PMS case control studies have shown higher, lower and similar concentrations of progesterone and Allo during the luteal phase. The consensus today is that the plasma concentration does not predict the PMDD diagnosis but, in sensitive patients, the levels appear to have importance.[9-11] The sensitivity of the $GABA_A$ receptor for Allo has been indicated as pathogenesis in PMDD. This is interesting because patients with PMDD have different sensitivity to Allo compared to controls[12] and serotonin reuptake inhibitors normalise neurosteroid sensitivity in patients with PMDD.[13] In a previous investigation of major depressive disorder, both Allo and Isoallo were measured in 11 patients.[14] The results showed a decrease in Allo concentrations and an increase in Isoallo concentrations in comparison with levels at recovery from major depression.[14] However, in patients with panic disorder, the same group found an increased Allo level and a decreased Isoallo level compared to controls.[15] Ten patients with panic disorder were also investigated during a sodium lactate induced panic attack and Allo and their Isoallo levels were compared with those of a matched control group. A decrease in Allo concentration and an increase in Isoallo concentration were noted during the attack compared to the control group.[16] The same group also investigated panic induction with high doses of cholecystokinin tetrapeptide in healthy volunteers. All volunteers displayed a pronounced panic reaction, although there was no change in either Allo or Isoallo plasma levels.[17]

Postpartum depression has been subject to neurosteroid investigations. It is well known that there is a major decrease in Allo levels at parturition when the production of Allo from the placenta ends. A therapy using i.v. injections of Allo has been approved by the Food and Drug Administration for use in humans and has shown efficiency in patients with postpartum depression.[18] A first preliminary clinical trial on treatment of major depressive disorder using an oral Allo derivate (SAGE-217) showed promising results[19] but a follow-up phase III study failed and the further development of SAGE-217 was paused, although not abandoned completely.[20]

3.2 | Menstrual migraine

In women with migraine, approximately 50% report an association between the occurrence of migraine attacks and menstrual cycle phase. Compared to the expected number of migraine attacks, women with menstrual migraine (MM) suffer from significantly more frequent attacks during the late luteal and peri-menstrual phase than during the follicular phase.[21,22] It is hypothesised that this increased incidence of attacks may be related to the withdrawal of the effects of Allo on the $GABA_A$ receptor. However, the data do not support the existence of a comorbidity between PMDD and menstrual migraine. In PMDD, a relief in symptoms during menstruation is always observed, whereas MM has its peak symptoms during menstruation.[23]

3.2.1 | Catamenial epilepsy

It has long been recognised that seizure frequency can vary with the menstrual cycle in women with epilepsy and with the oestrus cycle in animal models.[24,25] Herzog et al[26] identified three types of menstrual cycle–linked increase in seizure frequency from prospective seizure recordings in women with intractable complex partial seizures. Catamenial epilepsy is a form of epilepsy in women where there is a seizure-exacerbation during the menstrual phase in a subset of women.[24,26-28] In addition, a more recent prospective multicentre study of women with partial epilepsy showed a two-fold greater seizure occurrence on the first day of menstrual flow compared to the mid luteal days.[29] Overall, 39% of the women fulfilled criteria for catamenial epilepsy.[30] In ovulatory cycles, the predominant pattern was an increase of seizures during menstruation closely following the rapid decrease in plasma levels of Allo. The exacerbation of seizure frequency during menstruation could thus be a withdrawal effect similar to that seen when an antiepileptic drug is suddenly discontinued. In experimental studies, progesterone increases the electroshock seizure threshold in animals, protects against metrazol-induced seizures and decreases the frequency of interictal spikes from an epileptic focus.[31,32] Moreover, luteal phase serum concentrations of progesterone reduced inter-ictal spike frequency in four out of seven women with partial epilepsy, and the effect was dependent on the plasma progesterone binding. In this experimental study, women with the highest plasma binding rate did not respond.[33] The antiepileptic effect of progesterone is suggested to be mediated by its metabolite Allo.[34]

3.3 | Body movement disorders

Postural control could vary during the menstrual cycle, measured as the displacement area in a balance testing situation. Women with PMS had a significantly greater displacement area during the luteal phase compared to women without cyclical changes in one such study.[35] The incidence of sports-related injuries is one clinical aspect that seems to be related to the luteal phase of the menstrual cycle.[36] In other body movement disorders (eg, essential tremor), extra synaptic $GABA_A$ receptors have been implicated as regulators of the oscillatory activity. In patients with essential tremor, physical or mental stress exacerbates the tremor; for example, in acute stress situations, such as when giving an oral presentation.[37] During stress, the concentration of Allo in the brain and plasma increases to a concentration that has a physiological impact on $GABA_A$ receptor activity.[2,38] The results from a neuroimaging study indicated that there is an increased sensitivity to GABAergic stimulus in the cerebellar-thalamocortical pathway generating the tremor.[39]

Tourette's syndrome is a disorder presenting as different kinds of motor and phonic manifestations called tics, with the symptoms increasing during stress.[40] The influence of neuroactive steroids on the frequency of tics has been studied in both humans and animal models. Inhibition of the 5α-reductase enzyme reduces the number of tics.

5α-Reductase plays a central role in the enzymatic pathway from progesterone to Allo. In addition, Allo administration increases tic frequency.[41]

3.4 | Cognition and dementia

Here, we discuss the effects of acute and chronic stress on learning and memory in humans and rodents, as well as the effects in rodent dementia models and the risk of developing dementia in humans. Allo and tetrahydrodeoxycorticosterone (THDOC) are both positive $GABA_A$ receptor modulating steroids and they increase during stress.[2,38] An acute short exposure of Allo, such as in an acute stress situation, is known to decrease neural activity in the hippocampus of rats.[42] Allo inhibits learning in rats tested in the Morris water maze (MWM)[43-45] and inhibits episodic memory in humans.[46] Episodic memory is a type of memory that is disturbed early in patients diagnosed with Alzheimer's disease (AD).[47] Allo also inhibits long-term potentiation and cholinergic action in rat hippocampus.[48,49] Functional magnetic resonance imaging tudies in women show that progesterone and Allo impair memory by reducing the activity in brain regions related to memory formation and retrieval.[50] Other $GABA_A$ receptor agonists (eg, benzodiazepines,[51] barbiturates[52] and alcohol[53,54]) also impair memory and learning in humans, with a possible risk of permanent damage, although the risk with low and moderate alcohol consumption is under debate.[55]

Chronic exposure to positive $GABA_A$ receptor modulators appears to inhibit learning and memory. One condition where this happens is in humans with hepatic encephalopathy (HE), a complication of liver cirrhosis, and animal models of HE are examples of conditions with high brain concentration of Allo and increased GABAergic tone combined with decreased vigilance and learning, as well as memory disturbances.[5,56-58] An increased GABAergic tone is one of the main hypotheses for the neuropathology of HE.[59,60]

Overall, chronic exposure of positive $GABA_A$ receptor modulators or constant increased sensitivity to Allo are possible contributing factors in several human disorders; for example, cognitive symptoms during chronic stress, burn out syndrome and HE. In these situations, disorders with high GABAergic tone are linked to cognitive disturbances and a risk of developing dementia.[61-67] It has been shown that individuals who develop permanent memory and learning disturbances among chronically stressed persons are those with continuing high steroid levels for a prolonged period.[63]

Chronical exposure to Allo has been shown to induce memory and learning disturbances in wild-type mice,[43,44] and Allo in low stress levels accelerates the development of dementia in AD mice models.[68,69] However, intermittent administration of high single dosages of Allo, with at least weekly spaced dosing, increases the regeneration of neurons and improves learning and memory in an AD mouse model,[70-72] whereas more frequent dosing impairs cognition.[70] Thus, Allo can act as both a disturber and enhancer of memory function in AD animal models.

In wild-type mice, chronic Allo treatment at low-stress levels, for 5 months, produces a permanent deterioration in learning and memory especially in females.[73] In this experimental study, MWM testing was conducted 1 month after the end of treatment and removal of all Allo from the body. The deterioration in memory and learning was correlated with a decrease in hippocampal volume.[73]

Medroxyprogesterone-acetate (MPA) is a synthetic progestogenic steroid but also a positive $GABA_A$ receptor modulator (R Das, G Ragagnin, J Sjöstedt, M Johansson, D Haage, M Druzin, SO Johansson, T Bäckström, unpublished). MPA is administered in combination with oestrogen not only to postmenopausal women as a menopausal hormone therapy (MHT), but also to younger women for various reasons. In a large US based study of risk of developing dementia during MHT, the frequency of probable dementia (mainly AD) doubled after 5 years of continuous oestrogen and MPA treatment.[61] The increase in dementia frequency was not considered to be the result of ischaemic events but rather to a biological/hormonal factor, probably MPA itself.[74] Oestrogen alone did not increase the dementia risk in the same study.[75] MPA acts as a positive $GABA_A$ receptor modulator on the human $\alpha5\beta3\gamma2$ $GABA_A$ receptor subtype with a similar potency as Allo and THDOC (R Das, G Ragagnin, J Sjöstedt, M Johansson, D Haage, M Druzin, SO Johansson, T Bäckström, unpublished). In a manner similar to progesterone, MPA can induce anaesthesia when given to rats.[76]

3.5 | Food consumption and weight increase

Obesity is one of the major causes of poor health and the most important factor in development of obesity is the consumption of more calories than needed.[77] Still, the mechanism behind the many endogenous driving forces that contribute to over-eating is not well understood. However, GABAergic transmission has been shown to be of importance for regulation of food intake.[78,79] In humans, situations with elevated Allo levels are associated with increased food intake, eating disorders, overweight and obesity.[80] The importance of $GABA_A$ receptors in the regulation of food intake was investigated in an animal study where the $GABA_A$ receptor antagonist bicuculline inhibited food intake in hungry, food-deprived rats.[81] The brain areas of particular interest in this perspective are the hypothalamic arcuate nucleus and paraventricular nucleus.[78] Studies of benzodiazepine-induced food intake indicate that the most abundant α-subunits of the $GABA_A$ receptor in the arcuate nucleus and paraventricular nucleus are α3 and α2.[82] Allo in concentrations as low as 2 nM significantly increased the spontaneous postsynaptic current in single dissociated cells in both the arcuate nucleus and paraventricular nucleus.[83] Animal studies have shown that Allo increased meal size in a dose-dependent manner[84] and that calorie dense food was preferred compared to less palatable food.[85] Moreover, Allo compared to placebo increased body weight significantly in rats after five treatment days, and weight gain was highly correlated with increased food intake.[86]

In humans, there is a correlation between serum Allo concentration and uncontrolled eating in obese women with polycystic ovarian syndrome.[87] Overweight and obese women have a different

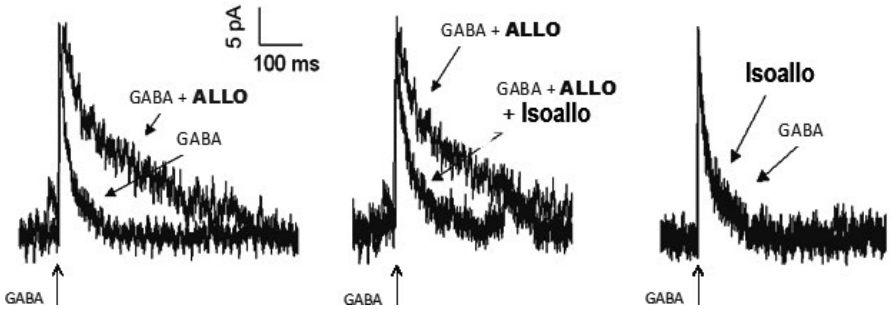

FIGURE 2 Isoallo normalises Allo induced enhancement of spontaneous GABA response at the $GABA_A$ receptor. Isoallo has no effect by itself. Spontaneous inhibitory postsynaptic currents (sIPSCs) were investigated by patch-clamp recordings in rat hypothalamic neurones from the medial preoptic nucleus (MPN). The marking GABA indicates spontaneous release of GABA. Left: typical traces of sIPSC under control conditions (EC solution) and traces when Allo is added to the media. Middle: effect of Allo compared with that seen when Allo + Isoallo is added to the media. Right: effect when Isoallo alone is added to the media. Reprinted with permission from Strömberg et al[90]

sensitivity to Allo compared to normal weight women, indicating that obesity changes the sensitivity to Allo.[88] Excessive weight-gain during pregnancy is related to high Allo levels compared to women with low weight-gain.[89]

In Prader-Willis syndrome, hyperphagia and obesity are key symptoms caused by a total lack of satiety sensations.[90] These patients have a deletion in chromosome 15 that contains genes coding for one $GABA_A$ receptor subtype.[90] Prader-Willis syndrome patients have a compensatory increased expression of α4 (12-fold), γ2 (5-fold), and α1 and α3 (>1.5-fold), as well as a higher density of $GABA_A$ receptors with the subunits α4, βx and δ visualised in neuroimaging studies.[91,92] Also, the α4, βx, δ receptor subtype is known to be hypersensitive to Allo.[93]

3.6 | Rational for treating positive $GABA_A$ receptor modulating steroid dependent disorders with $GABA_A$ modulating steroid antagonist (GAMSA)

A plausible hypothesis is that the symptoms and disorders discussed above are related to increased Allo concentrations in the brain or withdrawal effects after exposure to high Allo levels, with both mechanisms mediated via the $GABA_A$ receptor. In addition, the sensitivity to Allo exposure varies between individuals and may be increased by the up-regulation of more sensitive $GABA_A$ receptor subtypes. In this context, a modulation of the specific Allo effect on the $GABA_A$ receptor by a neurosteroid with antagonistic properties (ie, a GAMSA) would be beneficial, decreasing the negative symptoms.

4 | ANTAGONISM AGAINST POSITIVE $GABA_A$ RECEPTOR STEROID MODULATOR IN THE BRAIN

4.1 | In vitro studies

It is well known that the progesterone metabolites Allo (3α-hydroxy-5α-pregnan-20-one) and its isomer pregnanolone (Preg, 3α-hydroxy-5β-pregnan-20-one) enhance GABA action via their own binding sites on the $GABA_A$ receptor.[94] Their 3β-isomers, Isoallo (3β-hydroxy-5α-pregnan-20-one) and isopregnanolone (Isopreg, 3β-hydroxy-5β-pregnan-20-one) lack these effects on $GABA_A$ receptors.[95-98] Interactions between the 3β-isomers and the 3α compounds Allo and Preg, as well as their effect on action potentials, have been studied in slices from rat hippocampus in vitro. In these studies, population spikes (POPSP) after electrical stimulation of the CA1 stratum radiatum were measured in the stratum pyramidale. Allo and Preg inhibited the POPSP but Isoallo had no significant effect compared to the vehicle control. Increasing dosages of Isoallo caused a dose-dependent reduction of the Allo effect on POPSP. Full blockage was seen at a molar ratio of 1:1 and Isoallo also blocked Preg inhibition, but not muscimol inhibition, of POPSP.[99]

One effect of positive modulators of the $GABA_A$ receptor is the increase of GABA-mediated chloride ion (Cl^-) flux through this receptor. This is known to occur with several $GABA_A$ agonists such as benzodiazepines and barbiturates, as well as with Allo.[100,101] A previous study investigated the effect of Isoallo on GABA-mediated Cl^- uptake in cortical homogenates from adult rats. Isoallo inhibited Allo-induced Cl^- uptake; however, Isoallo up to the maximal dosage tested (1 mM) did not affect baseline Cl^- uptake in rat cortical homogenates. Neither did Isoallo interact with GABA, flunitrazepam or pentobarbital-induced increase of Cl^- uptake. These results indicate that Isoallo may be a useful functional blocker of positive $GABA_A$ receptor modulators similar to Allo when used at concentrations that do not affect baseline GABAergic neurotransmission.[102]

We have used patch-clamp recordings of spontaneous inhibitory postsynaptic currents (sIPSCs) to study different 3β-steroids in dissociated neuronal cells from rat hypothalamus. We tested the 3β-steroids together with Allo or singly without Allo for their ability to modulate the spontaneous GABA-mediated chloride flux. The Allo-enhanced GABA response was reduced by the tested 3β-steroids. Figure 2 shows the decay time after a spontaneous GABA release in absence and in the presence of Allo. The $GABA_A$ receptor's opening time was prolonged in the presence of Allo. This prolonged open time induced by Allo was inhibited and normalised with Isoallo, although Isoallo alone had no effect on the sIPSCs. Two of

the 3β-steroids however, potentiated GABA-evoked chloride ion uptake and prolonged the sIPSCs decay time in the absence of Allo. The other three steroids tested, including Isoallo, had little or no effect on GABA induced receptor opening. These results suggest that some 3β-steroids may be used as therapeutics and act by counteracting the negative effects of Allo.[103,104]

4.2 | In vivo animal studies

The interaction of Isoallo with Allo induced anaesthesia was studied in male rats using burst suppression of the electroencephalography for 1 second (silent second) as the threshold criteria for deep anaesthesia. Pre-treatment with i.v. Isoallo for 2 minutes prior to a threshold test with i.v. Allo increased the Allo amount needed in a dose-dependent manner. When Allo and Isoallo were infused simultaneously at three molar ratios, a linear increase in the threshold dose of Allo was seen in relation to the dose of Isoallo. Thus, Isoallo could antagonise the anesthetic action of Allo in these experiments.[105]

In a mouse model of Tourette's syndrome, Allo exacerbated the syndrome.[27] Because the main target of Allo is the $GABA_A$ receptor, it could be hypothesised that an Allo antagonist such as Isoallo may mitigate the symptoms. A model of stress enhanced tic frequency was used in a well-established Tourette's syndrome model (ie the D1CT-7 mice model). The Allo antagonist Isoallo, when applied dose-dependently, reduced the number of tic-like manifestations during stress in D1CT-7 mice.[106]

As shown above, Allo could impair learning and memory in animals.[43] In another study, a new GAMSA (3β-20β-dihydroxy-5α-pregnane) was evaluated as a blocker of Allo-induced effects on memory and learning of rats in vivo and of chloride ion uptake into hippocampal microsacs in vitro. The time taken by the rats to find the hidden platform in the MWM was significantly reduced in the group injected with Allo + 3β-20β-dihydroxy-5α-pregnane compared to the Allo-injected group. The rats injected with 3β-20β-dihydroxy-5α-pregnane located the platform as fast as the placebo treated controls. In line with these findings, chloride ion uptake to GABA-ergic neurones increased with Allo + GABA but was hindered by 3β-20β-dihydroxy-5α-pregnane.[107] Similar antagonistic effects towards Allo induction were seen with pregnenolone sulphate (PS) treatment.[44] PS acts as a GABA antagonist, whereas the 3β-hydroxy-steroids only antagonise the Allo effect. PS is therefore more dangerous because it can cause seizures similar to bicuculline.[108] PS has different channel properties compared to the 3β-hydroxy-steroids.[109] In another study, PS infused into the rat basal magnocellular nucleus enhanced memory performance, whereas Allo disrupted memory.[45]

A new GAMSA, a 3β-OH compound for oral administration, called GR3027, has been synthesised and tested both in vitro and in animal studies at our laboratory.[110] GR3027, selectively antagonises the enhanced activation of $GABA_A$ receptors by neurosteroids such as Allo and THDOC. In patients with HE, cognitive and motor impairment is very common and a major cause of disability in HE patients. Hyperactivation of $GABA_A$ receptors and elevated Allo levels are involved in the pathogenesis. Two different animal HE models have been used with the aim of assessing whether GR3027 improves motor coordination and spatial learning. The HE models were (i) rats with chronic hyperammonemia as a result of ammonia feeding and (ii) rats with portacaval shunts. Motor coordination was assessed via beam walking and spatial learning and memory were assessed in the MWM and the radial maze. In both hyperammonemic and portacaval shunts rats, GR3027 restored motor coordination and spatial memory and learning.[110]

4.3 | Human studies

As noted above, Allo exerts an anesthetic and sedative/hypnotic effect through potentiation of GABA at the $GABA_A$ receptor.[105,111] The acute effect of Allo could be measured in the saccadic eye velocity (SEV) model. SEV is aparameter strongly controlled by GABAergic transmission and comprises an objective evaluation of a pharmacological, sedative effect of an administered compound. In healthy women, SEV and subjective ratings of sedation have been used to evaluate the pharmacodynamic response to Allo. In these studies, i.v. administered Allo decreased SEV and increased sedation scores and the effect correlated with serum concentrations of Allo.[111] Furthermore, using repeated measurements of SEV and self-rated sedation, similar studies have investigated whether Isoallo could antagonise Allo-induced effects in healthy female volunteers. In a single-blind cross-over design, 12 women were investigated on three separate occasions and were given Allo alone or Allo in combination with one out of two Isoallo doses. The effect of Allo was counteracted by simultaneous Isoallo administration. The antagonistic effect of Isoallo was detected at half the dosage of Allo. It was thus concluded that Isoallo antagonised the Allo-induced effects on SEV and self-reported sedation[112] (Figure 3).

In the first clinical trial, Isoallo was used in a s.c. preparation called Sepranolone (Asarina Pharma). The disorder treated was PMDD. Sepranolone was given as a luteal phase treatment in a randomised, parallel-group, placebo-controlled study design. The results obtained showed that Sepranolone reduced PMDD symptoms significantly better than placebo based on the per protocol analysis. The effect was better in the population treated as intended compared to those starting and ending the treatment too early, which suggests that Sepranolone needs to be present in appropriate serum levels during the late luteal phase to exert the best possible effect. It was concluded that the results constituted an initial indication that Sepranolone could reduce symptom severity and impairment in PMDD more efficiently than placebo.[113]

Golexanolone (GR3027) (Umecrine Cognition) is a novel oral GAMSA developed specifically for the treatment of cognitive and vigilance impairment as a result of allosteric over-activation of $GABA_A$ receptors. From animal studies, the compound GR3027 has been shown to counteract the effects of Allo and has therefore been considered as a possible future pharmaceutical product for humans suffering from Allo-related over-activation of $GABA_A$ receptors. The

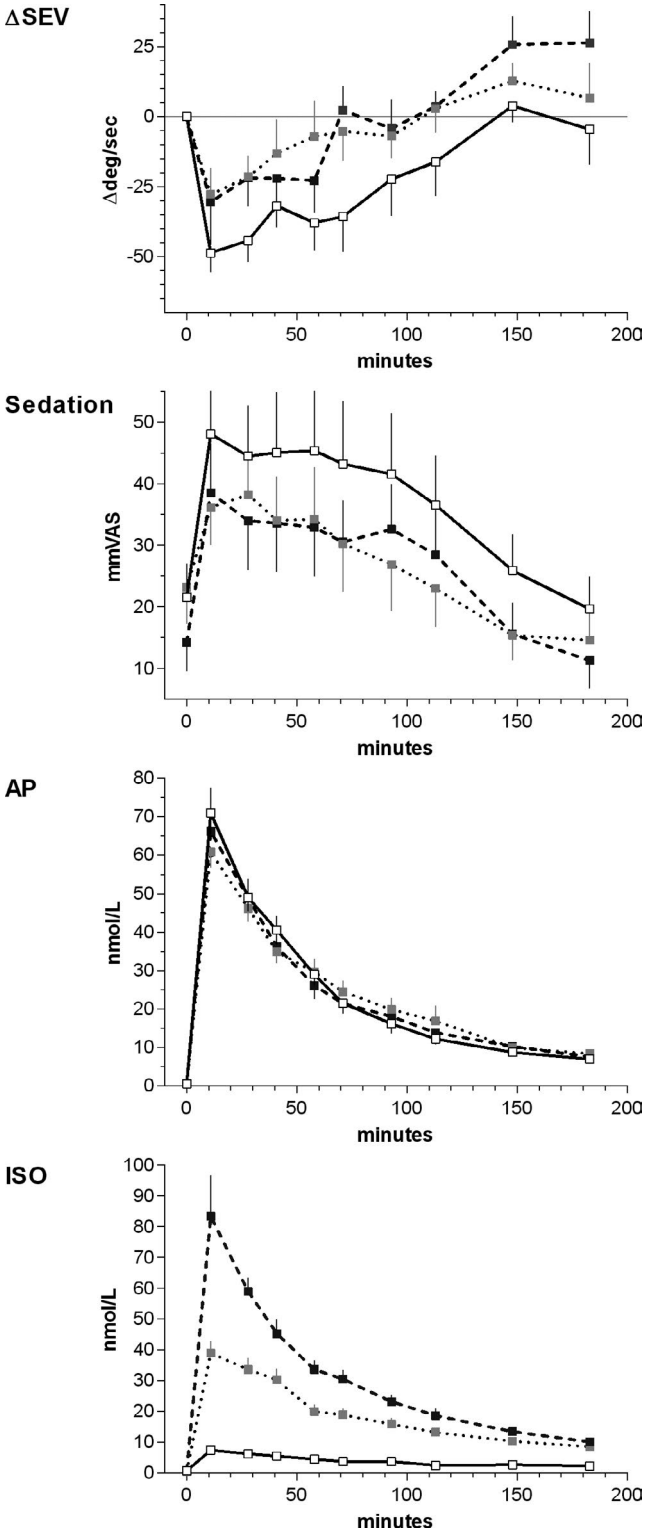

FIGURE 3 Change in the same subjects in saccadic eye velocity (SEV), self-rated sedation, Allo (AP) and Isoallo (ISO) serum levels following i.v. administration of Allo alone (white squares; n = 12), Allo:Isoallo low (light grey squares; n = 12) or Allo:Isoallo high (dark grey squares; n = 11), respectively. Data are given as the mean ± SEM. Both doses of AP + ISO had a significantly smaller reduction in SEV compared to AP alone. Allo:Isoallo low, but not Allo:Isoallo high, had a significant reduction in sedation compared to Allo alone. Reprinted with permission from Bengtsson et al[99]

GR3027-mediated antagonistic effect on the Allo-mediated reduction of SEV and increased self-rated sedation have been studied in a double-blind, placebo-controlled, cross-over study. GR3027 inhibited the Allo-induced decrease in SEV and Allo-induced sedation. Thus, the oral compound GR3027 could mitigate the inhibition of brain function induced by Allo at well tolerated doses.[114]

In a recent study, the effects of Golexanolone on the electroencephalogram, subjective sleepiness and cognitive performance of adult patients with HE were investigated. Patients with Child-Pugh A/B cirrhosis and an abnormal continuous reaction time were randomly dosed over 3 weeks with Golexanolone or placebo. Psychometric hepatic encephalopathy score, animal naming test, Epworth Sleepiness Scale and electroencephalogram (mean dominant frequency; delta + theta/alpha + beta ratio) were obtained at baseline, as well as after 10 and 21 days of treatment. Golexanolone treatment resulted in favorable changes vs. placebo in Epworth Sleepiness Scale, mean dominant frequency and delta + theta/alpha + beta ratio. Post hoc analyses showed an improvement in continuous reaction time, psychometric hepatic encephalopathy score and animal naming test, which suggests an efficacy signal by cognitive measures as well.[115]

5 | CONCLUSIONS

Hyperactivity or an increased GABAergic tone as a result of either exposure to positive allosteric $GABA_A$ receptor modulating steroids, or increased sensitivity to Allo, is related to several symptoms and disorders. Treatment with $GABA_A$ modulating steroid antagonists appears to mitigate the severity of the symptoms and disorders.

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CONFLICT OF INTERESTS

TB has shares in Umecrine AB, Umecrine Cognition AB and Asarina Pharma AB. RD is employed by Umecrine AB.

AUTHOR CONTRIBUTIONS

Torbjörn Bäckström: Conceptualisation; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualisation; Writing-original draft; Writing – review & editing. **Roshni Das:** Conceptualisation; Data curation; Investigation; Methodology; Writing – review & editing. **Marie Bixo:** Conceptualisation; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing – review & editing.

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