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$GABA_A$ receptor modulating steroid antagonists (GAMSA) are functional *in vivo*

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ABSTRACT

$GABA_A$ receptor modulating steroid antagonists (GAMSA) selectively inhibit neurosteroid-mediated enhancement of GABA-evoked currents at the $GABA_A$ receptor. 3α-hydroxy-neurosteroids, notably allopregnanolone and tetrahydrodeoxycorticosterone (THDOC), potentiate $GABA_A$ receptor-mediated currents. On the contrary, various 3β-hydroxy-steroids antagonize this positive neurosteroid-mediated modulation. Importantly, GAMSAs are specific antagonists of the positive neurosteroid-modulation of the receptor and do not inhibit GABA-evoked currents.

Allopregnanolone and THDOC have both negative and positive actions. Allopregnanolone can impair encoding/consolidation and retrieval of memories. Chronic administration of a physiological allopregnanolone concentration reduces cognition in mice models of Alzheimer's disease. In humans an allopregnanolone challenge impairs episodic memory and in hepatic encephalopathy cognitive deficits are accompanied by increased brain ammonia and allopregnanolone. Hippocampal slices react *in vitro* to ammonia by allopregnanolone synthesis in CA1 neurons, which blocks long-term potentiation (LTP). Thus, allopregnanolone may impair learning and memory by interfering with hippocampal LTP. Contrary, pharmacological treatment with allopregnanolone can promote neurogenesis and positively influence learning and memory of trace eye-blink conditioning in mice.

In rat the GAMSA UC1011 inhibits an allopregnanolone-induced learning impairment and the GAMSA GR3027 restores learning and motor coordination in rats with hepatic encephalopathy. In addition, the GAMSA isoallopregnanolone antagonizes allopregnanolone-induced anesthesia in rats, and in humans it antagonizes allopregnanolone-induced sedation and reductions in saccadic eye velocity. 17PA is also an effective GAMSA *in vivo*, as it antagonizes allopregnanolone-induced anesthesia and spinal analgesia in rats. *In vitro* the allopregnanolone/THDOC-increased GABA-mediated $GABA_A$ receptor activity is antagonized by isoallopregnanolone, UC1011, GR3027 and 17PA, while the effect of GABA itself is not affected.

Contents

Abbreviations: AD, Alzheimer's disease; GAMSA, $GABA_A$ receptor modulating steroid antagonists; EEG, electroencephalography; HE, hepatic encephalopathy; i.p., intraperitoneal; i.v., intravenous; LTP, long term potentiation; 17PA, (3α5α)-17-phenylandrost-16-en-3-ol; SEV, maximal saccadic eye velocity; s.c., subcutaneous; sIPSC, spontaneous postsynaptic current; THDOC, tetrahydrodeoxycorticosterone; TSPO, mitochondrial translocator protein; UC1011, 3β-20β-dihydroxy-5α-pregnane.

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1. Neurosteroid-enhancement of GABA-evoked currents

GABA is the major inhibitory neurotransmitter within the central nervous system. It exerts effects by activation of neuronal membrane-bound $GABA_A$ receptors, thereby opening the receptor chloride channel and in mature neurons chloride usually flows into the neuron and hyperpolarizes the cell, thereby reducing excitability. Endogenous steroids, including allopregnanolone (3α-hydroxy-5α-pregnan-20-one) and THDOC (3α,5α-tetrahydrodeoxycorticosterone) are positive allosteric $GABA_A$ receptor modulators [45],Fig. 1. They both act via the same two specific binding sites on the receptor [32,31], thereby increasing the inhibitory tone. Allopregnanolone is formed from progesterone, and THDOC from deoxycorticosterone, via reactions catalyzed by 5α-reductase followed by 3α-hydroxysteroid dehydrogenase, which are both present in the brain [47]. Under some conditions, e.g. stress, at least allopregnanolone is synthesized locally within the brain [54].

In hippocampal neurons allopregnanolone increases both the peak amplitude and duration of chloride currents induced by GABA [45]. While in acutely isolated neurons from the medial preoptic nucleus allopregnanolone dramatically prolongs the GABA-mediated spontaneous postsynaptic current (sIPSC), probably by reducing rates of GABA unbinding from the receptor [29]. In such preparations allopregnanolone also seems to have presynaptic effects, increasing the frequency of spontaneous GABA release and thereby increasing the frequency of sIPSCs [30].

THDOC also increases the GABAergic tone by increasing membrane expression of the α4 $GABA_A$ receptor subunit [1]. In this study it was shown that THDOC potentiates the protein kinase C-dependent phosphorylation of S443 within the α4 subunit, and in parallel enhances the insertion of receptors with this subunit into the membrane. The cell surface levels of receptors with α1 or α5 subunits where not increased. THDOC by this mechanism selectively increases tonic inhibition as α4-$GABA_A$ receptors are located extrasynaptically.

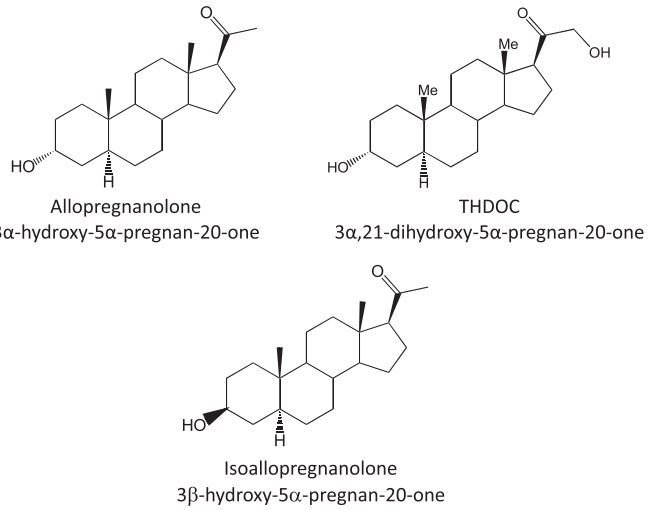

Fig. 1. Structures of the endogenous $GABA_A$ receptor positive allosteric modulators allopregnanolone and THDOC and the GAMSA isoallopregnanolone.

2. Learning and memory impairments by allopregnanolone and THDOC

2.1. Morris water maze tests of rats

The spatial version of the Morris water maze test is used to study hippocampal-dependent learning and acquisition of long-term spatial memory in rodents. Acute administration of allopregnanolone just before testing abolishes rats' capacity to learn the position of the hidden platform [36,65], Fig. 2. In the study by [36] control rats treated with vehicle quickly learned to complete the task, significantly improving during the first days and then maintaining a mean latency to the platform of around 40 s. In contrast, the latency of rats treated with allopregnanolone declined very little, remaining longer than 90 s on the sixth day of learning. This indicates that the rats did not learn the position of the platform, but occasionally found it by chance, thereby reducing the mean latency from the maximal 120 s per swim trail. The dose of allopregnanolone used to affect learning this profoundly was pharmacological (i.e. 2 mg/kg i.v.), and there was a high brain allopregnanolone concentration during the swim trials with a hippocampal concentration of 2 μmol/kg at the start of the learning session.

Retrieval of memory of the platform's position is also affected by acute administration of allopregnanolone. This has been demonstrated by studies in which rats learnt the platform position during four or six days in trials without allopregnanolone treatment. Rats subsequently treated with allopregnanolone, s.c. 17 mg/kg or 20 mg/kg, used a longer path than controls to swim to the platform [46,20]. However, in a non-spatial version of the test memory retrieval was not affected in the allopregnanolone-treated rats and the concentration of allopregnanolone was not reported [46].

THDOC also impairs rodent learning (5 mg/kg i.p., exposure not reported), as demonstrated by rats' performance in the Morris water maze using a matching-to-place paradigm [58]. The cited study showed that THDOC disrupts concept learning, working memory, and reference memory in rats selectively bred to be seizure-resistant. Interestingly, it also showed that THDOC administration marginally improved learning and memory in seizure-prone rats, which normally learn less well. These rats are known to express different $GABA_A$ receptor subunits in some brain areas [52], which may be involved in the different cognitive effects of THDOC.

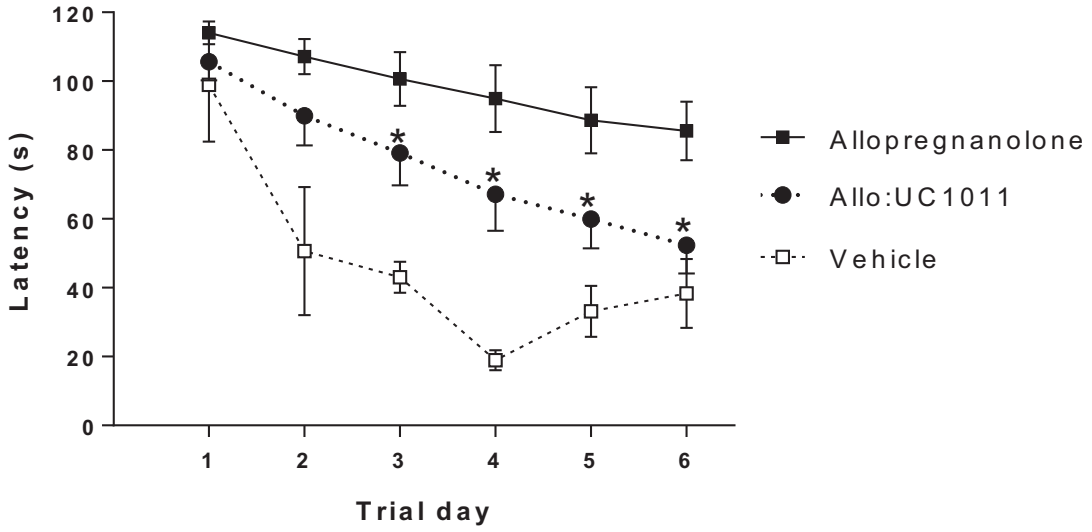

Fig. 2. The GAMSA UC1011 inhibits the impairment of learning in the Morris water maze test induced by allopregnanolone in rats, as manifested by the times rats took to swim to the platform (latency) after daily injections of 2 mg/kg allopregnanolone, 2 mg/kg allopregnanolone and 20 mg/kg UC1011, or vehicle—10% (2-hydroxypropyl)-β-cyclodextrin—eight minutes before the start of swimming.
(adapted from Turkmen et al., 2007)

2.2. Other learning and memory tests in rodents

More than 15 years ago, Reddy and Kulkarni showed that allopregnanolone (0.25 mg/kg s.c.) impairs rats' performance in the plus-maze learning task (the time a test animal spends moving from the end of one of the open arms to a "safe" covered arm in an elevated maze) [56]. Rapid completion requires use of spatial long-term memory, but may also be promoted by anxiety induced by heights and open spaces. Similarly to benzodiazepines, allopregnanolone has anxiolytic effects [57], thus reductions in anxiety may contribute to allopregnanolone-treated rats' slowness in reaching the "safety" offered by the closed arm. Allopregnanolone (10 mg/kg i.p.) also disrupts hippocampus-dependent contextual learning in C57BL/6J mice [22]. This study involved pre-exposure to a conditioning chamber to specifically target the focal process without interfering with pain sensitivity or behavioral performance.

Recently, it was shown that allopregnanolone apparently impairs both encoding and consolidation of an object memory [55]. First they administered allopregnanolone to male C57BL/6J mice i.p. either before or immediately after the first exploratory session of a novel object recognition task. Treatment with allopregnanolone 10 mg/kg or higher at either time point resulted in no discrimination of the novel object in the test situation 24 h later, i.e. mice did not remember the familiar object. Further studies showed that when allopregnanolone (0.1 μg/side) was micro infused bilaterally into the CA1 region of the dorsal hippocampus immediately following the object exploratory session the consolidation of the object memory was similarly impaired. Systemic allopregnanolone (10 mg/kg i.p.) also impaired encoding of a contextual memory when administered prior to the first session in the context test. The settings of these tests were non-spatial, hence allopregnanolone inhibited the formation and/or storage of non-spatial memory via effects on hippocampal neurons.

Chronic allopregnanolone administration with continuous exposure from an s.c. implanted Alzet® osmotic pump also affects learning and memory in mice models of Alzheimer's disease (AD) [10,9]. Results of cognitive tests reported in these studies indicate that such exposure advances development of Alzheimer's signs, increased levels of soluble beta-amyloid and impaired cognition, in the mice. In this setting the hippocampal allopregnanolone concentration was approximately 15 nmol/kg, a physiological concentration close to that found in rats after acute stress [54].

In sharp contrast, intermittent weekly treatment of AD mice with a pharmacological allopregnanolone dose induces neurogenesis through excitation of neural progenitor cells, thereby promoting neural proliferation and reducing AD pathology [33], this is further discussed below. In contrast to mature neurons, allopregnanolone-induced activation of the $GABA_A$ receptor complex in neural stem cells leads to depolarization.

2.3. Cognition in humans

Allopregnanolone exposure also affects memory in humans [38]. These authors gave single doses of allopregnanolone (0.07 mg/kg i.v., 100 nM in plasma) to women, who participated in memory tests pre- and post- injection. In this setting episodic memory (verbal recall) was impaired by the substance, relative to both their pre-injection performance and when vehicle treatment was received. However, their working memory and semantic memory were not affected.

There are also indications that allopregnanolone is involved in hepatic encephalopathy (HE), a debilitating cognitive and psychiatric condition caused by decreased liver function. Cognitive impairment in these patients decreases their quality of life and affects their daily living [51,8]. More specifically even early in the disease's progression spatial working memory is hampered [41] and both the learning capacity and short-term memory are impaired [70]. In addition, low grade HE associated with cognitive impairment and diagnosed by abnormal electroencephalography (EEG) and psychometric testing predicts the development of more severe episodic HE [50]. There is a substantial body of evidence to suggest that neurosteroids and increased GABAergic tone are central to the neuropathology in HE [2]. In a landmark study by Ahboucha et al. [3,4] elevated allopregnanolone concentrations in the brain (frontal cortex, the only brain region analyzed) of cirrhotic patients with HE that died in coma was shown, relative to both cirrhotic patients without HE and controls with neither liver disease nor psychiatric disorder [4]. Thus, increased levels of allopregnanolone in the brain were exclusively detected in the patients with HE. The mitochondrial translocator protein (TSPO) is

involved in an early step of neurosteroid synthesis, and TSPO binding sites are up-regulated in both human and experimental HE [2]. Even in living cirrhotic patients with mild-to-moderate HE binding of the TSPO ligand [^{11}C] (*R*)-PK11195 is increased in the brain [17]. However, no causal link between cognitive impairments in HE patients and increases in allopregnanolone levels in the brain has been established, as yet at least, while in rat models of HE/hyperammonemia increases in GABAergic activity are known to be involved in the cognitive function [18,27]. More importantly, recently a GAMSA was shown to improve cognitive and motor functions in two rat models of HE (see below [37]). Increased brain ammonia and neuroinflammation are also involved in the development of the neuronal dysfunction in HE [24].

This review focuses on negative consequences of neurosteroids that are positive modulators of the $GABA_A$ receptor and the possibility to block these negative effects with GAMSA. However, not all studies show that allopregnanolone negatively affects cognition, and similarly for progesterone that is the precursor of allopregnanolone. In a recent review concerning effects of steroids on learning and memory it was concluded that delivery of progesterone to women seldom influence cognitive performance positively and more often negatively [21]. We also believe that cognitive dysfunctions caused by allopregnanolone are a concern that possibly can be prevented by the use of GAMSA.

2.4. Long term potentiation can be inhibited by allopregnanolone

There are several indications that allopregnanolone might interfere with memory formation by inhibiting long-term potentiation (LTP) [64,35], a form of synaptic plasticity associated with memory processing. Notably, its synthesis in CA1 hippocampal neurons contributes to inhibition of LTP formation at certain conditions. Thus, application of either ethanol or ammonia to rat hippocampal slices both induces allopregnanolone formation in CA1 neurons and blocks LTP, and when 5α-reductase is inhibited with finasteride the allopregnanolone synthesis is blocked and LTP can be induced in the presence of ammonia [64,35]. The antibody used to detect neurosteroids in these studies are specific for 3α-hydroxy steroids, and predominantly binds to allopregnanolone, but other 3α-hydroxy steroids might also be recognized [53]. In hepatic encephalopathy the concentrations of ammonia and allopregnanolone are increased in the brain, which may block LTP and hence memory processes. Interestingly, finasteride improves motor-, EEG, and cellular changes in a rat model of HE and prevents the development of hepatic coma [49].

3. Pharmacological allopregnanolone treatments

Contrary to the studies described above treatment with allopregnanolone can be advantageous for specific cognitive performance and can induce neurogenesis and cell survival. In a series of experiments it has been shown that pharmacologic allopregnanolone treatment of the triple transgenic mouse model of AD (3xTgAD) can be beneficial.

Wang et al. in 2010 showed that the decreased neurogenesis in the subgranular zone of the hippocampal dentate gyrus in these AD mice was reversed by one allopregnanolone injection (10 mg/kg s.c.) [66]. In the same study it was shown that the memory of a trace eye-blink conditioning returned to normal in the 3xTgAD mice three weeks after a single allopregnanolone treatment. The reported cortex allopregnanolone concentration was high, i.e. 0.5 μmol/kg (159 ng/g) at half an hour [33,34] and still 47 nmol/kg (15 ng/g) 24 h after the treatment [66]. In further studies it was shown that for maximal efficacy treatment with allopregnanolone (10 mg/kg s.c.) should be once a week to promote neurogenesis, survival of the newly generated cells, and reduction in beta-amyloid [19]. It was in this study also shown that an intense treatment, i.e. three times a week for three month reduced neurogenesis in the AD mice. Singh et al. in 2011 followed up with a study showing that allopregnanolone (10 mg/kg s.c.) effectively influenced trace eye-blink conditioning in 6 and 9 month old 3xTgAD mice, but not in the 12 month old AD mice. Contrary, in 15 month old non-transgenic mice decreased learning of trace eye-blink conditioning and decreased neurogenesis was rescued by one allopregnanolone treatment [60].

To induce these positive effects by allopregnanolone the temporal exposure is very different to that by Bengtsson et al. that showed allopregnanolone to negatively affect the AD disease progression in two different AD mice models ($APP_{Swe}PS1_{\Delta E9}$ and $APP_{Swe/Arc}$ [10,9]). In those studies there was for several month a constant exposure to a low allopregnanolone concentration, i.e. 15 nmol/kg in the hippocampus. As described above, to induce neurogenesis the optimal administration schedule is to give allopregnanolone with one week intervals and at therapeutic doses the peak brain allopregnanolone concentrations is at least 0.5 μmol/kg that then quickly returns to basal levels [34]. Interestingly, allopregnanolone treatment is in progress as a therapeutic approach to regenerate the neurogenic capacity of the brain (ClinicalTrials.gov Identifier:NCT02221622).

There are also other examples where positive effects of allopregnanolone treatment have been shown in different animal models e.g. anxiolytic activity and decrease in epileptic discharges

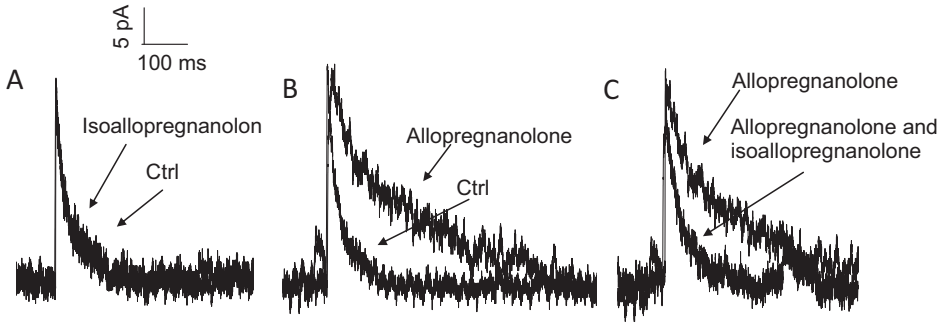

Fig. 3. The GAMSA isoallopregnanolone decreases the allopregnanolone-prolonged sIPSC while there is no interference when allopregnanolone has not been added. Traces shown are from one acutely isolated medial preoptic rat neuron. (A) Traces under control condition and with applied isoallopregnanolone are interchangeable, i.e. there is no antagonism of the sIPSC when allopregnanolone has not been applied, (B) Allopregnanolone prolongation of the sIPSC decay time, (C) Isoallopregnanolone antagonize the allopregnanolone-induced prolongation of the sIPSC.
(adopted from Strömberg et al., 2006)

[7,40,12,71,16]. There are also several recent reviews discussing treatment effects of allopregnanolone [14,44,15,28], and it is out of the scope of this review concerning GAMSA to go through these studies.

4. $GABA_A$ receptor modulating steroid antagonists (GAMSA)—*in vitro* studies

GAMSAs are antagonists to the positive $GABA_A$ receptor-modulating steroids. An example is the endogenous steroid isoallopregnanolone (3β-hydroxy-5α-pregnan-20-one, Fig 1), an epimer to allopregnanolone, structurally differing only in the orientation of the hydroxyl group at carbon 3 of the steroid A-ring. Several other 3β-hydroxy-steroids are also functional GAMSAs [68,61].

The antagonistic effect of isoallopregnanolone has been studied using several techniques, both *in vitro* and *in vivo*. Voltage-clamp studies of recombinant $GABA_A$ receptors containing rat α1-subunits expressed in *Xenopus* oocytes have demonstrated that isoallopregnanolone inhibits the receptors' potentiation by allopregnanolone [68]. Similarly, local application of isoallopregnanolone to the pyramidal cell layer of hippocampal slices from rats dose-dependently antagonizes allopregnanolone-mediated inhibition of the CA1 pyramidal neuronal population spike evoked by electrical stimulation of the Schaffer collaterals [67,69].

Isoallopregnanolone antagonism was first shown to be specific for steroids that positively modulate $GABA_A$ receptors by demonstrations that it antagonizes allopregnanolone-enhanced chloride ion uptake by isolated rat brain cortical microsacs in the presence of GABA, but not when the chloride ion uptake is driven solely by GABA [43]. No antagonism was detected when the benzodiazepine flunitrazepam or the barbiturate pentobarbital were used to enhance the chloride ion uptake either. More specific studies (Fig. 3A) have shown that isoallopregnanolone does not affect the sIPSC in acutely isolated neurons from the medial preoptic nucleus in the absence of allopregnanolone, i.e. when the current is induced solely by spontaneous release of GABA from presynaptic adhering nerve terminals. However, isoallopregnanolone abolishes prolongation of the sIPSC induced by allopregnanolone application (Fig. 3B and C, modified from Strömberg et al.) [61].

Other examples of GAMSA are UC1011 (3β-20β-dihydroxy-5α-pregnane) that in studies with isolated microsacs antagonizes the allopregnanolone potentiation of chloride ion uptake in the presence of GABA in both hippocampal and cortical microsacs [65], and GR3027 that antagonizes THDOC but not GABA at both human α1β2γ2 and α5β3γ2 $GABA_A$ receptors expressed in HEK-293 cells [37]. Finally, 17PA ((3α5α)-17-phenylandrost-16-en-3-ol) selectively antagonizes the 5α-reduced neurosteroid potentiation of the GABA response from rat α1β2γ2 $GABA_A$ receptors expressed in *Xenopus* oocytes [48]. This means that 17PA at the $GABA_A$ receptors antagonizes the effects of allopregnanolone, but not that of pregnanolone that is the 5β isomer of allopregnanolone. 17PA also antagonizes 5α-reduced steroids at native $GABA_A$ receptors in isolated rat hippocampal neurons and cortical synaptoneurosomes [48,39].

5. $GABA_A$ receptor modulating steroid antagonists (GAMSA)—*in vivo* studies

5.1. The GAMSA UC1011 is an inhibitor of allopregnanolone-decreased learning in rat

A direct evidence that GAMSAs may effectively antagonize neurosteroid-induced learning impairment is the demonstration by [65] that UC1011 antagonizes the impairment of rats' performance in the Morris water maze test induced by acute pre-administration of allopregnanolone (Fig. 2) [65]. Rats given allopregnanolone alone did not learn the platform's location, but the latency of rats given both allopregnanolone and UC1011 decreased during practice, and their performance was significantly enhanced by the GAMSA treatment during days 3–6 of the test. UC1011 alone had no effect on the rats' learning.

In the Morris water maze test a hippocampal-dependent memory is formed and allopregnanolone probably affects learning and memory via hippocampal $GABA_A$ receptors, while UC1011 probably enhances learning in allopregnanolone-treated rats by antagonistic effects within the hippocampus.

5.2. The GAMSA GR3027 restores learning and motor coordination in rats with hepatic encephalopathy

Recently this year it was shown that chronic daily treatment with GR3027 to rats with hepatic encephalopathy induced by chronic ammonia feeding or by portacaval shunts restores affected behaviors in the HE rats [37]. In both HE models an impaired motor coordination was recognized in the beam walking test, and GR3027 restored the motor function to that of controls. Also, in the Morris water maze test memory was significantly decreased in the HE rats, and treatment with GR3027 restored the impaired memory. Further, working memory was studied with the radial arm maze and in the test GR3027 restored the increased number of working errors found in the vehicle treated HE rats. At the same time the blood ammonia concentration was unaffected by GR3027 exposure.

The only known mechanism of GR3027 is to antagonize allopregnanolone and THDOC at the $GABA_A$ receptor. This point to the possibility that increased concentrations of positive $GABA_A$ receptor-modulating steroids are the cause, or at least part of the cause, of the disrupted CNS function seen in hepatic encephalopathy.

Of importance is also that the long term daily treatment with the GAMSA GR3027 did not negatively affect the rats in any sense, i.e. there were no toxicity signs.

5.3. In vivo *antagonism by isoallopregnanolone*

Isoallopregnanolone is also a functional GAMSA *in vivo*, in both rats [6] and humans [11], but no studies of its effects on learning or memory have been published.

A study of a threshold model of deep allopregnanolone-induced anesthesia in rat showed that when isoallopregnanolone was given either before or together with allopregnanolone the dose of allopregnanolone needed to reach the threshold increased. Thus, isoallopregnanolone antagonizes the anesthetic action of allopregnanolone in rats [6].

Intriguingly, allopregnanolone induces anxiety in pubertal female mice, but it is anxiolytic in pre-pubertal mice [59]. Similar changes in anxiety levels have been indicated by the time rats have spent in the open arms of an elevated plus maze following induction of endogenous allopregnanolone production by preceding restraint stress or allopregnanolone injection. However, pre-administration of the GAMSA isoallopregnanolone inhibited both the stress-induced anxiety in pubertal female mice and stress-induced anxiolytic effect of allopregnanolone observed in pre-pubertal female mice. Blocking stress-induced allopregnanolone production by finasteride had the same effects. These findings clearly indicate that isoallopregnanolone can antagonize both anxiety and anxiolysis induced by endogenous allopregnanolone.

In humans allopregnanolone dose-dependently decreases the maximal velocity of saccadic eye movement (SEV) and increases sedation [63]. SEV has been validated as a biomarker for effects of $GABA_A$ receptor modulators, especially benzodiazepines [23]. Administration of isoallopregnanolone together with

allopregnanolone reportedly diminishes the change in SEV and self-rated sedation, and the effects indicate that the antagonism is probably non-competitive [11]. Also, in a recent phase I/II clinical trial with isoallopregnanolone to treat premenstrual dysphoric disorder no adverse signs were noted by the GAMSA treatment [13].

5.4. In vivo *antagonism by 17PA*

In tadpoles 17PA antagonizes 5α-reduced steroid-induced anesthesia, while anesthesia induced by a 5β-reduced steroid was unaffected [48]. Also in rats sedation/hypnosis induced by allopregnanolone was antagonized by 17PA given intracerebroventricular [39]. This was analyzed by studies of the righting reflex, i.e. the ability of the rat to right when placed in a supine position. 5α-reduced steroids induce a loss of the righting reflex and when 17PA was present higher doses of the steroid were needed to lose the reflex. Also, with 17PA pretreatment the total sleep time after allopregnanolone was attenuated.

In rats allopregnanolone got a spinal analgesic action, and a dose-dependent analgesia has been measured by thermal threshold [62]. In this situation intrathecal 17PA completely prevented the analgesic allopregnanolone effect both on the normal and the inflammation evoked hyperalgesic paw [62].

6. Not all antagonists at $GABA_A$ receptors antagonize allopregnanolone and THDOC

Several studies have found that the benzodiazepine antagonist flumazenil does not antagonize steroids that positively modulate the $GABA_A$ receptor. For example, it has no effect on allopregnanolone's potentiation of the $GABA_A$ receptor current according to patch-clamp studies of hippocampal cultures [3], and *in vivo* it does not antagonize allopregnanolone-induced hyperphagia in rats [56], or pregnanolone discriminative stimulus effects in Rhesus monkeys [26]. Similarly, in humans flumazenil does not antagonize the reduction in saccadic eye velocity induced by allopregnanolone [5]. However, at least one study has reported an antagonistic effect: that flumazenil antagonizes allopregnanolone-decreased probe-burying in female Wistar rats, and thus apparently counters allopregnanolone's anxiolytic effect in them [25].

The ethanol antidote Ro15-4513 is a weak benzodiazepine antagonist that *in vivo* does not antagonize the contextual learning disruption caused by allopregnanolone in C57Bl6 mice [22]. However, *in vitro* the allopregnanolone potentiation of the $GABA_A$ receptor current has been shown to be antagonized [3].

In contrast, $GABA_A$ receptor antagonists directed against the GABA site, like bicuculline, or chloride channel blockers such as picrotoxin, or the $GABA_A$ receptor negative allosteric modulator pregnenolone sulfate block both the effect of GABA and allopregnanolone/THDOC potentiation of the receptor [45,42,29]. Use of such substances *in vivo* is dangerous as blockage of the GABA effect at the $GABA_A$ receptor leads to over-excitation with induction of seizures. This highlights the need to use GAMSAs, i.e. specific neurosteroid antagonists that do not affect GABA responses, when attempting to inhibit cognitive deficits or other adverse conditions mediated by allopregnanolone, for safety reasons.

7. Conclusion

The purpose of this review article was to describe a novel class of compounds as potential therapeutic agents to prevent the actions of neurosteroids on the $GABA_A$ receptor.

Neurosteroids such as allopregnanolone and THDOC are positive allosteric modulators of $GABA_A$ receptors with pleiotropic physiological manifestations. For example, pharmacologic treatment with allopregnanolone can be beneficial to block Status Epilepticus and with special treatment schedules neurogenesis can be induced in rodents. However, allopregnanolone and THDOC also impair cognitive functions in rodents and in humans.

Changes in the levels of neurosteroids have also been associated with clinical symptoms. For example increased brain levels of allopregnanolone offer a cogent explanation for the notion of 'increased GABAergic tone' in hepatic encephalopathy (HE) and the increased GABAergic tone is a credible cause of the cognitive symptoms that appears in HE. In premenstrual dysphoric disorder (PMDD) plasma allopregnanolone levels are increased in the luteal phase of the menstrual cycle, and in the same time period the PMDD symptoms are present.

Pharmacological approaches using agents that inhibit the positive modulatory action of GABA-steroids on the $GABA_A$ receptor could therefore offer new therapeutic tools for the management and treatment of HE in patients with liver disease and PMDD in women.

GAMSA is a novel class of pharmacologically active compounds that are antagonists to positive $GABA_A$ receptor modulating steroids. GAMSA antagonizes the neurosteroid-potentiation of the GABA mediated activation of the receptor without affecting the GABA induced chloride flux through the receptor. In conclusion, GAMSA offers a novel pharmacological approach by antagonizing the enhanced GABAergic tone caused by increased levels of $GABA_A$ receptor modulating steroids and with a favorable side effect profile supported by preclinical and clinical use.

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