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ABSTRACT

Objective/Hypothesis: Although now considered to be the most effective treatment for post traumatic stress disorder (PTSD), extinctionbased therapies require substantial time and investment for both the patient and provider, averaging 10 sessions or more of approximately 1h each to achieve significant beneficial effects. Thus, treatments that enhance the efficacy of extinction therapies and reduce the number of required sessions for remission would be of great benefit. Ideally, such adjunctive treatments may reduce the need for long term medication. Preclinical studies have demonstrated that glutamate transmission in the amygdala is necessary for long term extinction of fear memories. Furthermore, d-cycloserine (DCS), a partial NMDA receptor agonist acting on the glycine modulator site, significantly enhances fear extinction (fear extinction). DCS treatment has also been shown to significantly enhance efficacy of extinction-based therapy across a number of anxiety disorders. However, efficacy of DCS may be limited, as its effects diminish over repeated dosing and it is not effective in all subjects or protocols. Here we will examine the efficacy of 2 novel classes of compounds which enhance glutamate signal to facilitate fear extinction. First, we will examine the efficacy of Org-24598, a glycine transporter 1 (GLYT1) inhibitor to increase fear extinction. GLYT1 inhibition has been shown to facilitate glutamate transmission in limbic regions that modulate emotional processes, and are more efficacious in facilitating glutamate signal than DCS. Second we will examine the efficacy of CX546, a positive allosteric modulator of AMPA receptors to increase fear extinction. **Methods:** To assess the effects of these compounds on fear extinction, we proposed to use the FPS model of fear conditioning and extinction in mice. We will compare dose responses of both compounds to vehicle controls in their ability to facilitate fear extinction and examine if these effects were maintained with repeated testing. These initial studies characterizing and comparing the longevity of our test compounds on fear extinction will be important to inform clinical studies of the relative utility of these compounds to facilitate extinction-based therapies. **Results:** Our preliminary results in the mouse model of fear extinction showed that unlike in rats, DCS, the positive control, does not enhance fear extinction. We thus switched to utilization of the FPS model in rats which has been shown previously to be sensitive to DCS. We found that DCS significantly enhanced fear extinction as previously reported in rats, indicating we had established a protocol sensitive to fear extinction enhancement by glutamatergic drugs. The GLYT1 inhibitor Org-24598 (3, 10 mg/kg) significantly increased fear extinction in rats. Unlike the GLYT1 inhibitor, the AMPAKINE CX546 (3, 30 mg/kg) did not affect fear extinction. **Conclusions:** These data indicate that the GLYT1 inhibitor Org-24598 but not the AMPAKINE CX546 facilitates fear extinction similar to DCS.

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Introduction

Exposure therapy, a fear extinction based treatment, has been shown to be effective in treating post traumatic stress disorder (PTSD). Exposure-based therapies require substantial time and investment for both the patient and provider, averaging 10 sessions or more of approximately 1 h each to achieve significant beneficial effects. Thus, treatments that enhance the efficacy of extinction therapies and reduce the number of required sessions for remission would be of great benefit. Ideally, such therapy strategies may reduce the need for long term medication. This proposal uses a preclinical animal model of fear learning and extinction (fear potentiated startle) to test the efficacy of two novel compounds that enhance glutamate signaling. Previous reports indicate that the partial glutamate receptor agonist D-cycloserine (DCS) has been shown to facilitate animal models of extinction which has translated into recent clinical reports of efficacy in anxiety disorders when administered during extinction based psychotherapies. DCS however, has been shown to have some limitations in both dosing and efficacy in some circumstances however (Norberg, Krystal, & Tolin, 2008). Here we will examine the efficacy of glycine transporter (GLYT1) inhibition and positive allosteric modulation of AMPA receptors in facilitation fear extinction. GLYT1 inhibitors are reported to show significantly greater enhancement of glutamate signaling compared to DCS (Sur and Kinney, 2007), as well as facilitate glutamate transmission in limbic regions that modulate emotional processes. We will examine the efficacy of treatment with a glycine transporter inhibitor during extinction training to enhance fear extinction retention and reduce fear reinstatement in mice. We will also examine CX546, an “ampakine” in the class of AMPA receptor positive allosteric modulators, which enhances molecular markers of learning in the cortex and hippocampus (e.g. long term potentiation) and enhance learning in rodents and humans (Arai and Kessler, 2007). These studies will provide information either in support or against further research of these compounds to increase fear extinction. Over this reporting period we have validated the animal model used to detect glutamate signaling using the positive control DCS, as well as tested the glycine transporter inhibitor Org-24598.

Main Body

The objective of this proposal is to test the efficacy of two novel classes of glutamate system enhancing compounds, ampakines and glycine transporter inhibitors, to facilitate fear extinction learning. *Rationale:* Rothbaum and Davis (2003) describe PTSD as a disorder characterized by a “failure of fear extinction after trauma”. In animals and humans, a conditioned fear association occurs when a conditioned stimulus (CS) and an aversive unconditioned stimulus (US) are presented in close temporal proximity. Thus the subject learns that the CS “predicts” the occurrence of the US. In the case of PTSD, environmental cues during trauma are associated with the pain and fear of the traumatic event, and these cues continue to evoke strong fear reactions long after the initial trauma has receded. In the laboratory this phenomenon is modeled in humans and animals by pairing a tone or light with noxious stimuli such as an electrical shock. Once the association between the CS and US has been learned, the presentation of the CS alone will invoke a conditioned fear response (e.g. autonomic activation, exaggerated startle response, avoidance behavior). The phenomenon of fear extinction occurs when the learned CS is then presented without the occurrence of the US, hence the subject learns that the CS no longer predicts the presence of the US and subsequent fear responses to the CS are inhibited. It is this phenomenon that is hypothesized to be disrupted in PTSD patients, which continue to show pronounced signs of anxiety, avoidance, and arousal in response to trauma reminders. Preclinical studies have demonstrated that glutamate transmission in the amygdala is necessary for fear extinction, as measured by extinction of fear potentiated startle (FPS; (for review see Myers and Davis 2006)). Furthermore, DCS, a partial NMDA receptor agonist acting on the glycine modulator site, significantly enhances fear extinction. Compared to controls, rats treated with d-cycloserine during fear extinction training show (1) greater reductions in fear post training, (2) generalized inhibition of other conditioned fear cues and (3) more resilient fear extinction when exposed to subsequent trauma (e.g. foot shock reinstatement). These studies have recently been translated into the clinic in two phobia populations, acrophobia and social phobia, who received DCS treatment during a type of extinction training (Norberg et al. 2008). DCS treatment significantly enhanced the extinction therapy effects on measures of phobia-specific and generalized anxiety compared to placebo treatment. For example, those taking DCS during therapy exhibited greater general improvement of anxiety symptoms, increased self exposure to CSs outside of therapy, and reduced autonomic measures of fear during CS presentation. These studies indicate that enhancement of glutamatergic transmission improves fear extinction in both animals and humans (for review see Myers and Davis 2006).

Hypothesis: Ligands that enhance glutamate transmission facilitate fear extinction (fear extinction) learning. To test our hypothesis, we proposed to examine the effects of 2 glutamate signaling enhancing drugs, a glycine transporter inhibitor (Org-24598) and a positive modulator of AMPA receptor activity (CX546) in ability to enhance fear extinction learning as measured by enhanced extinction of fear potentiated startle (FPS) in mice.

FPS: To assess the effects of these compounds on fear extinction, we used the FPS model of fear conditioning and extinction in rodents (Risbrough et al 2003). This assay has construct, face, and predictive validity for fear learning processes in humans. When rodents are presented with a CS previously paired with a shock US, acoustic startle responding is exaggerated compared to baseline (i.e. fear-potentiated startle). After initial fear learning, if rodents are subsequently presented with the CS without the US, they slowly extinguish the conditioned fear response to the CS. Thus, after fear extinction training, FPS is reduced. Hence FPS levels post extinction learning can be used as a measure of fear extinction.

1. Model validation: Prove that the assay being used to detect efficacy of glutamate signaling in fear extinction is sensitive to the positive control compound, D-cycloserine.

Expt.1. *Rationale:* To test these compounds in their ability to enhance fear extinction we first examined the sensitivity of our mouse fear potentiated startle extinction assay to detect efficacy of glutamate signaling enhancers, using DCS. Although these studies were not expressly delineated in the SOW, we had concerns that if we saw negative effects of the novel compounds tested, we would not be sure if it was due to a problem with the assay to detect positive efficacy in facilitating fear extinction. Thus we added DCS as a positive control in our initial studies. We used DCS as our positive control as it has proven efficacy in human studies of fear extinction therapy across a wide number of anxiety disorders (Norberg et al. 2008). We first wanted to be sure that our model detects

this positive control, supporting the use of the assay to measure efficacy of novel compounds to increase extinction. We had proposed to use the mouse model of fear potentiated startle to examine the efficacy of glutamate enhancing ligands to increase fear extinction. Mice were trained over 2 days to associate a tone CS (4 kHz, 30 s) with a mild footshock (0.4 mA, 10 training trials/day). After associative learning, mice were tested for learned fear of the tone CS by comparing their startle reactivity with and without the cue present (100-110 dB pulses with and without the presence of the tone CS, 30-120 sec intertrial interval, 12 trials of each type). Mice that exhibited significant learning of the cue (showed higher startle reactivity in the presence of the cue compared to when the cue was not present) went on to the extinction training day. For extinction training, mice were presented with 30 cue trials without a shock. Thirty min before extinction training mice were treated with vehicle or DCS (1-30 mg/kg, i.p.). Twenty four hours later, mice were tested for FPS. DCS treatment had no effect

Figure 1. DCS treatment has no effect on FPS extinction in mice.

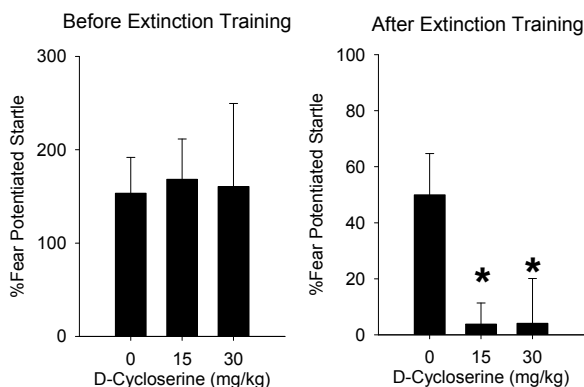
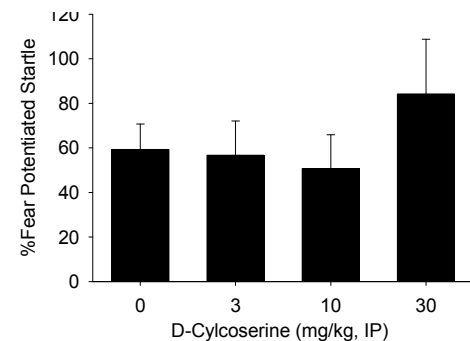


Figure 2. DCS facilitates fear extinction of FPS in rats. Left panel: Fear potentiated startle before extinction testing, drug groups were matched for FPS levels before drug administration to ensure no baseline differences in fear memory. Right panel: DCS was administered during extinction training, and FPS was tested 24 hours later. Main effect of drug $F(2,27)=4.46$, $p<0.05$. * $p<0.05$ vs. vehicle, Tukey post hoc test.

on fear extinction in mice (Figure 1). Further studies using different parameters and dose ranges were unsuccessful (data not shown). Indeed, in some experiments we found DCS treatment *decreased* fear extinction (e.g. interrupted extinction learning resulting in higher fear; data not shown). We attempted 4 variations of the mouse fear potentiated startle assay as well as used alternate methods to examine fear learning (freezing instead of acoustic startle) to detect a DCS effect of fear extinction, but were unsuccessful in detecting a positive effect. Because we could not develop an assay that was sensitive to the positive controls, DCS, we decided to establish the rat model of FPS in the laboratory which has been reported by others to be sensitive to DCS of fear extinction (Walker et al. 2002). Using the same protocol as reported by (Walker & Davis, 2002), we found that DCS treatment during extinction training in rats significantly increased the amount of fear extinction (Figure 2) measured 24 hours after drug treatment. Hence the rat FPS assay was deemed suitable for use to examine the effects of novel glutamate signaling enhancers on fear extinction.

2. Aim 1: Test the hypothesis that fear extinction is enhanced by glycine transporter 1 inhibition.

A. Test the hypothesis that Org-24598 induces facilitation of extinction training. The glycine transporter inhibitor Org-24598 has been shown to induce increased glycine signaling in the forebrain (see Appendix A) at a dose of 10 mg/kg. Based on this information our first study was to investigate the effects of 3 and 10 mg/kg treatment 60 min before extinction training. As shown in Figure 3, we found a significant effect of Org-24598 treatment to enhance fear extinction in rats. Following this positive effect we then conducted an experiment to examine if Org-24598 treatment is as effective using fewer training trials. Studies in humans indicate that DCS effects to enhance extinction are critically dependent on the number of training trials given while under DCS treatment, for example too few trials during treatment will render DCS ineffective (Norberg et al. 2008). Our

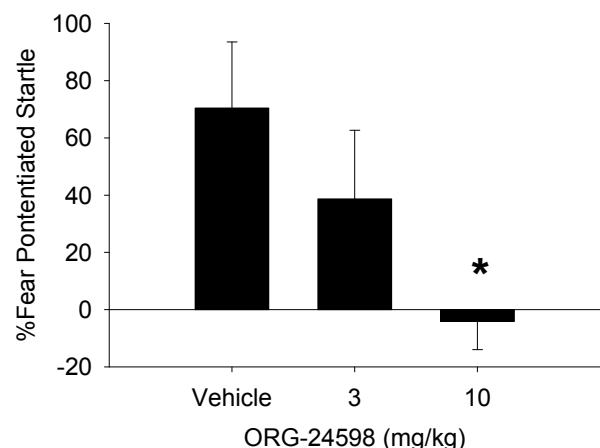


Figure 3. Org-24598 dose dependently facilitates fear extinction. Main effect of drug $(2,26)=4.14$, $p<0.05$, * $p<0.05$ vs. Vehicle, Tukey's post hoc test.

initial studies were using 30 training trials over 1 day. To examine if Org-24598 was as effective using fewer trials, we tested the ability of 10 mg/kg Org-24598 to facilitate extinction learning using 20 trials. We found a non-significant reduction in %FPS with 20 extinction training trials compared to vehicle (Mean $\pm$ SEM %FPS: Vehicle=74 $\pm$ 31, Org-24598=56 $\pm$ 18, $F(1,20) < 1$, N.S.).

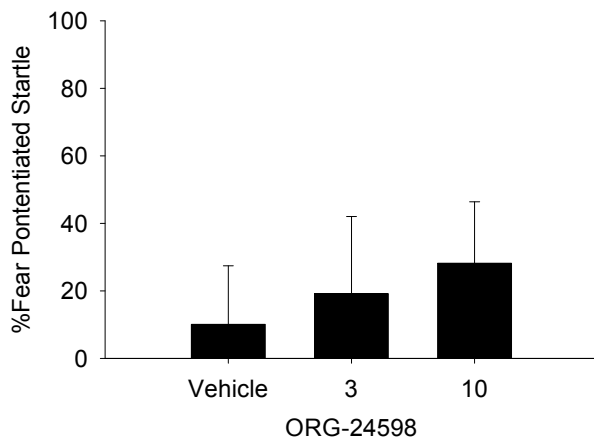


Figure 4. Fear extinction is stable 7 days post-extinction training across treatment groups.

extinction that was unaffected by drug treatment (data not shown).

C. Test the hypothesis that Org-24598 treatment blocks fear-reinstatement. *Rationale:* Another question in developing fear extinction-enhancing drugs for PTSD is if the drug can also provide greater protection against reinstatement of the fear responses. This application may be most

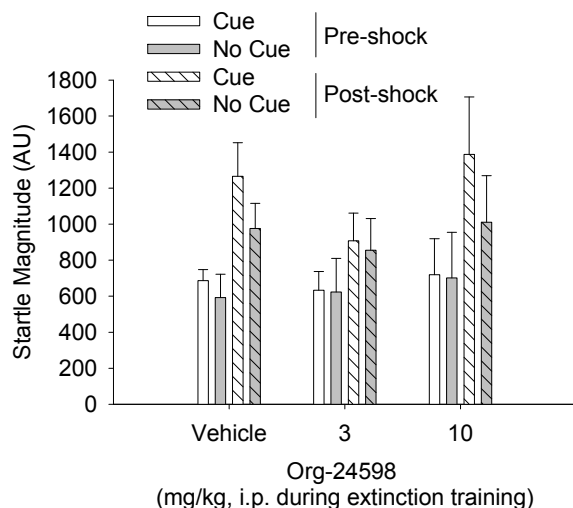


Figure 5. Fear re-instatement in rats treated with Org-24598 during extinction training.

important for those suffering PTSD from trauma that may happen again, for example in those in the military that are exposed to combat stress repeated times over the course of their active duty. A separate group of animals were treated with Org-24598 during extinction training (3 and 10 mg/kg). Seven days later, rats were exposed to a reinstatement session. This session consists of an initial block of 24 startle trials, half with the cue present (cue trials) and the other half without (no cue trials). As can be seen in Figure 5, rats showed no FPS across groups, indicating fear extinction had occurred. After this block, rats were presented with 1 US (0.6 mA) to reinstate fear of the cue. After the shock presentation a second block of cue and no cue startle trials was presented. The presentation of the US increased startle responding during the cue compared to testing before the US (Cue X shock interaction: $F(2,27)=4.02$, $p=0.055$). There was no significant effect of Org-24598

treatment to block this effect, however it appeared that the 3 dose may have a trend to reduce reinstatement. Future studies will examine a lower dose range for Org-24598.

3. Aim 2: Test the hypothesis that fear extinction is enhanced by AMPAKINE CX-546.

- Test the hypothesis that CX-546 induces facilitation of extinction training.** Unlike the GLYT1 inhibitor, the AMPAKINE CX-546 (Figure 6) did not facilitate extinction training. This lack of efficacy may be due to the poor bio-availability of CX546.
- Test the hypothesis that CX-546 facilitation of extinction training is long lasting and increase generalization.** As observed in the acute study, CX-546 did not have an effect on long lasting extinction levels (Figure 7), although there did appear to be a non-significant reduction in residual fear expression in the low dose treated group (3 mg/kg, Figure 7). CX-546 had not effect on generalization of extinction to the shock grid CS (data not shown).

C. Test the hypothesis that Org-24598 treatment blocks fear-reinstatement. CX-546 did not block reinstatement (data not shown).

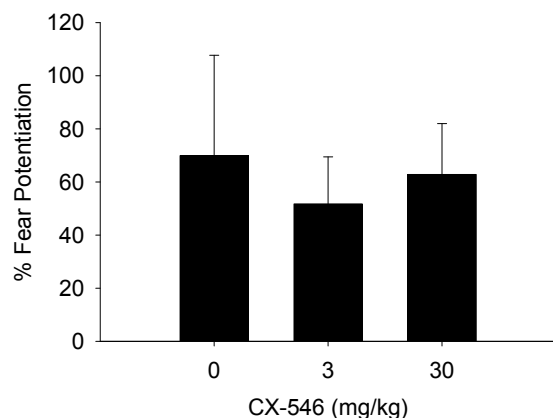


Figure 6. CX-546 treatment does not facilitate extinction learning in rats.

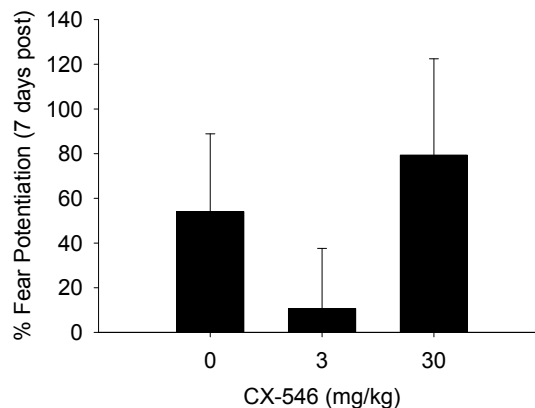


Figure 7. CX-546 treatment has no effect on longevity of extinction learning.

Key Research Accomplishments

- Successful validation of our pre-clinical model of fear extinction, finding that it is sensitive to the effects of DCS, a proven compound that facilitates fear extinction in humans. This finding is critical to the interpretation of our future findings using novel compounds in this protocol.
- Using our model, we found that the glycine transporter inhibitor Org-24598 shows dose dependent facilitation of extinction learning. Preliminary data indicate that extinction is retained with re-testing. It does not appear to block re-instatement, however further testing is required using higher doses to conclude if there is or is not efficacy in this task.
- CX-546, a positive modulator of AMPA receptors (AMPAKINE) did not show efficacy in any of the models tested. Other AMPAKINES with different pharmacodynamic properties may have efficacy however, as CX-546 has been reported in the literature to have relatively poor bioavailability compared to other proprietary AMPAKINES.
- Based on the validation of the model in the laboratory under the DOD funding we have established a collaboration with Cortex Pharmaceuticals, in which we will examine a number of their putative cognitive enhancing drugs for facilitation of extinction. These studies are ongoing and funded from other resources, and we plan on publishing the data presented here with the data from these new compounds. These data together will provide the field information on new possible drug targets for adjunctive treatments for exposure therapy in PTSD.

Reportable Outcomes

- These findings were presented at the Military Health Research Forum (MHRF) in September, 2009 (see poster in appendix B).
- These findings have resulted in a Material Transfer Agreement between Cortex and UCSD for our lab to further examine different AMPAKINE compounds for utility in reducing fear extinction (funded by the VA Center of Excellence for Stress and Mental Health).

Conclusions

Our assay is effective in examining facilitation of extinction. Thus far we have shown that Org-24598 is effective in facilitation extinction, however further study is required to confirm that fewer training trials are required for full extinction in Org-24598 rats compared to vehicle. Doses that are effective in facilitating extinction do not appear to block re-instatement, however higher doses may be needed to see such a dual effect. The implications of the research support the potential use of Org-24598 but not CX546 treatment for extinction therapies in humans. The lack of effect of CX546 does not indicate that all AMPAKINES would be ineffective in this model however (see Yamade et al. 2010 for facilitation of context extinction), and thus we are currently comparing the effects of AMPAKINES with

different pharmacodynamic properties at the receptor to determine if these compounds are effective in this assay.

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Effect of Org 24598 on Glycine Levels in Brain Regions of Freely Moving Rats



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Introduction

The uptake of glycine into presynaptic nerve terminals or the neighbouring glial processes may constitute an efficient mechanism by which the postsynaptic action of glycine can be terminated. This process is presumed to be carried out by two different glycine transporters, GlyT-1 and GlyT-2, which belong to the Na⁺- and Cl⁻-dependent neurotransmitter transporter superfamily. The GlyT-1 has a wide distribution throughout the CNS and three isoforms, GlyT-1a, b, c, have been identified. Some evidence has shown that the GlyT-1 may associate with NMDA receptors, whereas the GlyT-2 may associate with the strychnine sensitive glycine receptors. The present study investigates the effects of Org 24598, a selective GlyT-1 inhibitor, on amino acid levels in different brain regions of freely moving rats using the microdialysis technique.

Methods

Male rats (Wistar, 250-300 g, Harlan) were anaesthetised with a mixture (1:1) of Hypnorm and Hypnovel. A 15 mm long guide cannula was stereotactically inserted. At least 24 h after stereotactic insertion of the guide cannula, rats were immobilised and a custom-built microdialysis probe (4 mm AN69 dialysis membrane) was gently inserted into the hippocampus (final microdialysis probe location, mm, A-4.5, V-7.9, L-4.9), frontal cortex (final microdialysis probe location, mm, A-3.5, V-5.5, L-1.5) or striatum (final microdialysis probe location, mm, A-0.5, V-6.8, L-2.5). Each animal was placed in a single Plexiglas animal cage with free access to food and water. The probe was perfused with artificial cerebrospinal fluid (aCSF: mM, NaCl 120.6, KCl 2.40, KH₂PO₄ 0.49, MgCl₂ 1.28, CaCl₂ 1.10, NaHCO₃ 27.40, Na₂PO₄ 0.48, glucose 7.10, initial pH 7.4) at 2.0 µl/min. Org 24598 was dissolved in either aCSF and given locally (0.1 - 100 µM) or saline and given peripherally (10 mg/kg, i.p.). Dialysate amino acid levels were quantified immediately by high performance liquid chromatography coupled to a fluorescence detector.

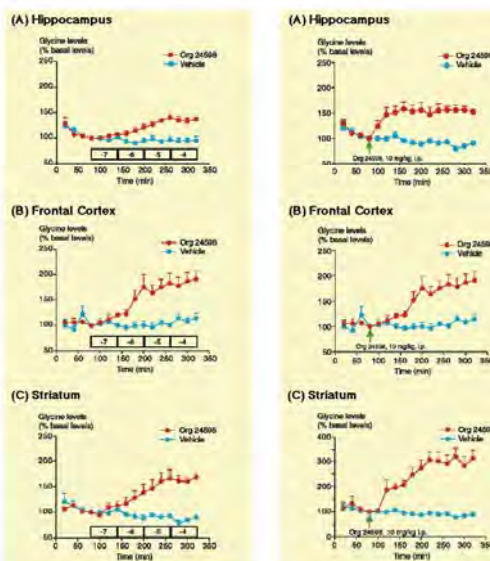


Figure 1. Effect of local administration of Org 24598 (0.1 - 100 µM) to modulate the glycine levels in (A) hippocampus, (B) frontal cortex and (C) striatum of freely moving rats. The glycine levels are expressed as a percentage of the levels at 80 min of measurements. The horizontal bars represent application of the drugs. Data represent the mean $\pm$ s.e.m., n=5-5.

Figure 2. Effect of systemic administration of Org 24598 (10 mg/kg, i.p.) to modulate the glycine levels in (A) hippocampus, (B) frontal cortex and (C) striatum of freely moving rats. The glycine levels are expressed as a percentage of the levels at 80 min of measurements. Data represent the mean $\pm$ s.e.m., n=4-5.

Summary of Results

- The basal levels of glycine were 62.3 ± 7.7 , 85.3 ± 6.5 and 76.9 ± 9.2 pmol/40 µl dialysates, in hippocampus, frontal cortex and striatum, respectively.
- The basal and Org 24598-induced increases in glycine levels are insensitive to tetrodotoxin (5.0 µM).
- Local application of Org 24598 (0.1 - 100 µM) concentration-dependently increased the glycine levels by 7-40 % in hippocampus, 20 - 90 % in frontal cortex and 13 - 70 % in striatum.
- Systemic administration of Org 24598 (10 mg/kg, i.p.) minimally increased the glycine levels by 6.5 % in 300 s above the basal levels in hippocampus, frontal cortex and striatum.
- Neither local nor systemic administration of Org 24598 modulated the levels of other amino acids such as aspartate, glutamate, serine, alanine, threonine, valine, leucine and GABA in these brain areas.

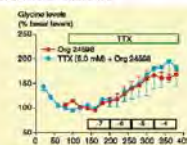


Figure 3. Effect of tetrodotoxin (5.0 µM, TTX) administered via the microdialysis probe on Org 24598 (0.1 - 100 µM)-induced increases in glycine levels in striatum of freely moving rats. The glycine levels are expressed as a percentage of the levels at 80 min of measurements. The horizontal bars represent application of the drugs. Data represent the mean $\pm$ s.e.m., n=5-5.

Discussion

- Org 24598 selectively increases glycine levels in various brain regions of freely moving rats by inhibition of the GlyT-1 transporter (Walker et al., this meeting, 68.3, 1999).
- The glycine measured in the present study are less likely to be neuronal in origin (TTX-insensitive) but more likely to be derived from other components such as glial cells (Zafra et al., Eur. J. Neurosci., 7, 1542, 1995).
- The Org 24598-induced increase in glycine may subsequently interact with the NMDA receptors to produce various physiological and pharmacological effects (e.g. Smith et al., Neurosci., 5, 927, 1992).

In Vitro Characterisation of Org 24598, a Selective Glycine Uptake Inhibitor.



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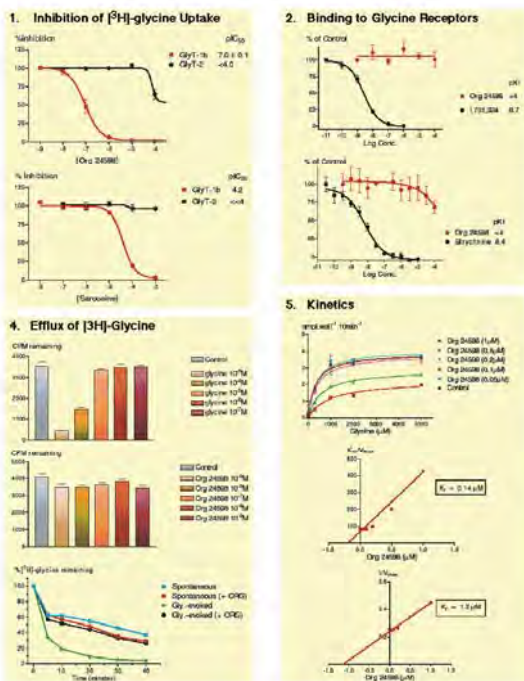
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Introduction

Alteration of glycine levels in the mammalian central nervous system may influence inhibitory activity mediated by the strychnine-sensitive glycine receptor (SSGR) or excitatory neurotransmission through the glycine receptor on the NMDA receptor complex. SSGRs are located predominantly in the spinal cord and brainstem and are closely associated with the neuronal GlyT-2 transporter, whereas GlyT-1 is distributed more widely in the CNS and may play a role in controlling glycine concentrations in the vicinity of NMDA receptors. GlyT-1 exists as 4 isoforms, designated a, b, c, and d, which arise from the use of alternate promoters or as splice variants. We describe here the *in vitro* characterisation of a selective GlyT-1 inhibitor.

Methods

- Glycine uptake assays were performed using CHO cells stably transfected with hGlyT-1b or hGlyT-2. Cells were grown in 96 well microtitre plates, washed with Hanks Balanced Salt Solution (HBSS) to remove culture medium then inhibition of [³H]-glycine uptake was determined in the presence of varying concentrations of Org 24598 or sarcosine.
- Radoligand binding experiments to assess interaction with SSGR, or the NMDA glycine coagonist site were performed using rat spine and brain membranes and [³H]-strychnine or [³H]-MDL105,519 respectively.
- Uptake assays for noradrenaline, dopamine, serotonin and GABA using synaptosomal preparations from rat brain and the corresponding [³H]-labelled transmitter.
- Potential for interaction with subtypes of dopamine, serotonin and noradrenaline receptors was assessed in radioligand binding experiments using heterologously expressed receptors or rat brain preparations.
- Efflux experiments were conducted by pre-loading cells expressing hGlyT-1b with [³H]-glycine for 30 minutes before exposure to cold glycine or Org 24598. Radioactivity remaining in the cell monolayer was then determined.
- Kinetics of inhibition were determined by constructing saturation curves in the presence of Org 24598 (0.01-100 µM). Resulting K_{0.5} and V_{max} values were used to derive estimates of the inhibition constants.

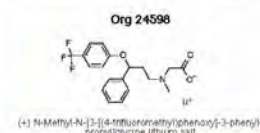


Summary of Results

- Org 24598 is a highly selective inhibitor of hGlyT-1b with negligible action at GlyT-2. The compound is approximately 600x more potent than sarcosine.
- Org 24598 does not show significant interaction with the NMDA glycine receptor as determined in binding studies using the radioligand [³H]-MDL105,519. Also, no appreciable displacement of [³H]-strychnine from rat spine membranes was noted.
- Isolated Na⁺/K⁺-dependent transporters were not inhibited and no significant affinity for a wide range of receptors was measured.
- Org 24598 does not act as a substrate for GlyT-1b and does not participate in hetero-exchange with glycine.
- Org 24598 shows a mixed inhibitory profile in this isolated system.

Discussion

- Selective inhibitors of related Na⁺/Cl⁻-dependent transporters have been shown to be clinically effective in a variety of neurological conditions involving depression and epilepsy.
- The possible association of GlyT-1 and NMDA receptors may afford an opportunity for inhibition of the transporter to enhance NMDA receptor function through elevated concentrations of the obligatory coagonist glycine.
- This mechanism may have relevance in addressing hypo-glutamatergic function associated with psychosis.



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Stress and Mental Health

- Exposure therapy, a form of psychotherapy based on fear extinction, is effective in treating post traumatic stress disorder (PTSD).
- Treatments that enhance the efficacy of extinction therapies and reduce the number of required sessions for remission would be beneficial to both the patient and provider.
- The partial glutamate receptor agonist D-cycloserine (DCS) increases extinction learning in rodent models and in humans. DCS has some limitations however in both dosing and efficacy in some circumstances (Norberg et al. 2008).
- Because glutamate signaling appears to be critical for fear extinction (Walker et al. 2002), we examined the efficacy of other drug classes that increase glutamate signaling to facilitate fear extinction learning in rats.
- Glycine transporter (GLYT1) inhibitors are reported to show significantly greater enhancement of glutamate signaling compared to DCS (Sur and Kinney, 2007).
- AMPA/kines are positive allosteric modulators of the AMPA receptor that increase learning and memory in rodents and humans (Arai and Kessler, 2007).

1. Fear potentiated startle is a classical conditioning procedure. During conditioning, a "neutral" stimulus (CS, e.g. light) is paired with an aversive stimulus (US, e.g. foot-shock). After conditioning, the light cue will elicit a fear-like state (conditioned response, CR) in the subject. The CR is measured in the form of exaggerated acoustic startle responding. Male Sprague Dawley rats were trained over 2 sessions.
2. 24 hrs after conditioning, rats were treated with vehicle or drug and then began extinction training (30 cue presentations, n=9-10/group).
3. 24 hrs after extinction training, rats were tested for fear potentiated startle.

1. Fear Conditioning:



2. Extinction Training:



3. Acoustic Startle Testing:



small startle response = "low fear"

large startle response = "high fear"

Figure 1 consists of two bar graphs. The left graph, titled 'BEFORE EXTINCTION TRAINING (PRE-TREATMENT)', shows % Fear Potentiated Startle on the y-axis (0 to 300) for three D-Cycloserine doses on the x-axis: 0, 15, and 30 mg/kg. The bars are black with error bars. The right graph, titled 'POST EXTINCTION TRAINING (POST-TREATMENT)', shows % Fear Potentiated Startle on the y-axis (0 to 100) for the same three doses. The bars are black with error bars. In the post-treatment graph, the bars for 15 and 30 mg/kg are significantly lower than the 0 mg/kg bar, indicated by asterisks (*).

Group	Dose (mg/kg)	% Fear Potentiated Startle (approx.)
BEFORE EXTINCTION TRAINING (PRE-TREATMENT)	0	155
	15	170
	30	160
POST EXTINCTION TRAINING (POST-TREATMENT)	0	50
	15	5
	30	5

% Fear Potentiated Startle

Group	% Fear Potentiated Startle
Vehicle	~70
3	~38
10	~-5*

ow fear^{II}

ORG-24598 (mg/kg)

Fear extinction remains stable 7 days post-test

Group	% Fear Potentiated Startle
Vehicle	~15
3	~25
10	~30

*p<0.05 vs. Vehicle control

CX546 (mg/kg)	% Fear Potentiated Startle
0	~70
3	~52
30	~55

The behavioral assay used was sensitive to DCS, supporting its validity for use in identifying drugs that facilitate fear extinction. Org-24598 increased fear learning, and this effect was stable over 1 week. Org-24598 treatment also tended to reduce reinstatement of conditioned fear (data not shown). These data support further research of GLYT1 inhibitor compounds as effective facilitators of fear extinction learning. Initial studies indicate that the AMPAKINE CX546 was not effective in facilitating extinction at doses that have been shown previously to increase learning in other cognitive tasks (Ng et al. 2009). These data suggest that fear extinction is enhanced only via increased activity of the NMDA and not AMPA receptor signaling. This hypothesis is supported by recent findings that GLYT1 inhibition increases NMDA over AMPA receptor signaling in the amygdala (Mao et al. 2009). Further studies will examine CX546 across a larger dose range, and examine the effects of both compounds to enhance generalization of fear extinction to non-extinguished fear cues.

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