Antinociceptive action of GLYX-13: an *N*-methyl-D-aspartate receptor glycine site partial agonist

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Inhibition of *N*-methyl-D-aspartate (NMDA)-mediated neurotransmission has been demonstrated to provide antinociceptive actions in a number of animal models of tonic and neuropathic pain. However, both competitive and noncompetitive NMDA receptor antagonists are ataxic at analgesic doses. Partial agonists and antagonists of the NMDA-associated glycine site have demonstrated antinociceptive actions at doses that are not ataxic. In this study, we present data showing that GLYX-13, an NMDA receptor, glycine-site, partial agonist, also is antinociceptive in the rat formalin model of tonic pain and in the rat constriction nerve injury model of neuropathic pain at doses not inducing ataxia. *NeuroReport* 19:1061–1063 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

The *N*-methyl-D-aspartate (NMDA) receptor–ionophore complex (NMDA receptor) plays a central role in modulating normal synaptic transmission, synaptic plasticity, and excitotoxicity in the central nervous system. Distinct NMDA receptor subtypes have been identified that differ in their sensitivity to a variety of ligands, kinetic properties, and interactions with intracellular proteins [1]. The NMDA receptor has been recognized as an attractive target for the development of a number of therapeutic compounds. However, preclinical and clinical studies with competitive and noncompetitive NMDA receptor antagonists have demonstrated very narrow therapeutic indices. Hence, researchers have investigated the allosteric glycine modulatory site as a potential target for the design of NMDA receptor modulators with a greater therapeutic index. Owing to the specificity of the glycine coagonist binding site, a number of glycine-site modulators have recently been characterized that do seem to have therapeutic potential [1]. Moreover, as suggested by Priestley *et al.* [2], partial agonists at this site may be better therapeutic candidates in that they would, as antagonists, allow normal synaptic transmission to take place while at the same time suppress receptor hyperactivity, and as weak agonists they may facilitate receptor activation without running the risk of overactivating the receptors. Several glycine receptor modulators have been found to improve mechanical allondynia in rat neuropathic pain models [1]. Of interest is gabapentin, a marketed drug used for treating neuropathic pain, which seems to induce analgesia via an NMDA-dependent mechanism. Specifically, the antinociceptive actions of this drug in the formalin assay are dose-dependently antagonized by D-serine, a prototype agonist of the NMDA-associated glycine receptor [3,4], although a recent report suggests that it may modulate voltage-gated calcium channels as well [5].

GLYX-13 is a tetrapeptide of amino acid sequence Thr–Pro–Pro–Thr and is derived from a monoclonal antibody (B6B21) directed against the glycine site of the ionotropic NMDA receptor. GLYX-13 is a partial agonist at the glycine site of the NMDA receptor in the central nervous system [6]. We undertook an evaluation of antinociceptive activity of GLYX-13 to determine if analgesia could be obtained in the absence of motor incoordination.

Methods

For the evaluation of the antinociceptive actions of GLYX-13 in the rat formalin assay [7], male Sprague–Dawley rats (125–170 g) were manually restrained for a subcutaneous injection of 1.5% formalin (50 µl with a 26 ga needle) into the lateral footpad on the plantar surface of the left hind paw. After formalin injections, rats were placed in individual clear plastic cylinders of 30 cm diameter. Drug effects on the second phase of the pain response were monitored with observations conducted over the period between 10 and 40 min after formalin injection. Each group was composed of 10 animals. Vehicle, GLYX-13, or gabapentin was administered subcutaneously at the nape of the neck 10 min before the formalin injection. The time spent licking or elevating the injected limb was quantitated over this 20 min observation period.

Next, GLYX-13 was evaluated in the chronic constriction nerve injury model of neuropathic pain [8]; 5 mg/kg GLYX-13 was used in these studies on the basis of earlier experiments showing that this concentration was an effective antagonist dose [6]. Male Sprague–Dawley rats (200–225 g) were anesthetized with sodium pentobarbitone (6 mg/kg, intraperitoneally) and supplemented as necessary with isoflurane (1–3% in oxygen). Under asceptic conditions, the right sciatic nerve was exposed by blunt dissection at the mid thigh level and 1 cm freed of adhering connective tissue. Four chromic catgut (4.0) ligatures were tied to lightly constrict the nerve at 1 mm intervals. The overlying muscle and skin were sutured and upon recovery from anesthesia the rats returned to cages of soft padded bedding and to cages with sawdust bedding after 24 h. At days 5, 7, 9, and 11 post recovery, mechanical allodynia was assessed with Von Frey filaments (calibration numbers, 3.61–6.10) applied to the plantar surface of the hind paw from below. The filaments were evaluated in ascending order with the threshold for both the ipsilateral and contralateral paws being evaluated. The withdrawal threshold was defined as the lowest force of two or more consecutive Von Frey filaments to elicit a withdrawal reflex. Only animals that developed mechanical allodynia (withdrawal response $\leq$5 g of force) in their nerve-injured paw by day 11 were utilized for drug testing.

Results

Both GLYX-13 and gabapentin demonstrated dose-dependent efficacy in the rat formalin model of tonic pain (Fig. 1). Gabapentin-treated rats were ataxic at the highest dose, whereas GLYX-13-treated rats were not ataxic at any of the doses examined (data not shown). No statistically significant differences in analgesic effects of GLYX-13 and gabapentin were observed.

The antinociceptive actions of vehicle or GLYX-13 (5 mg/kg) were evaluated at 15 and 60 min after dosing.

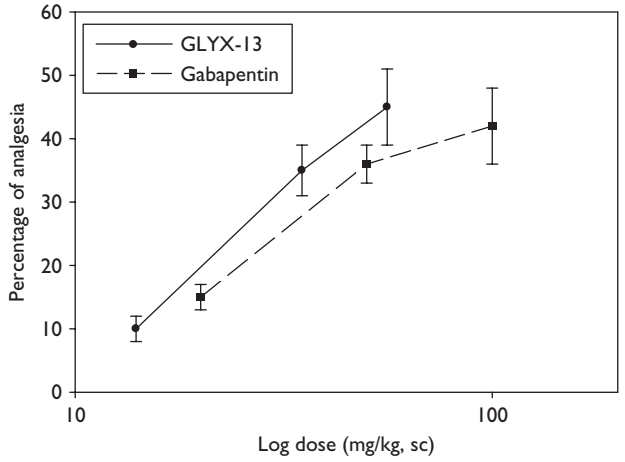

Fig. 1 Antinociceptive actions of GLYX-13 and gabapentin in the rat formalin model of tonic pain. Percentage analgesia is the percentage reduction (on the basis of the area under the curve for vehicle + formalin) in flinches in the late phase response (10–40 min) after intraplantar formalin injection (50 µl of 5% formalin) compared with control values arbitrarily set at 100%. Drugs were administered subcutaneously, 15 min before formalin. N=8–10 per group. Mean ± SEM.

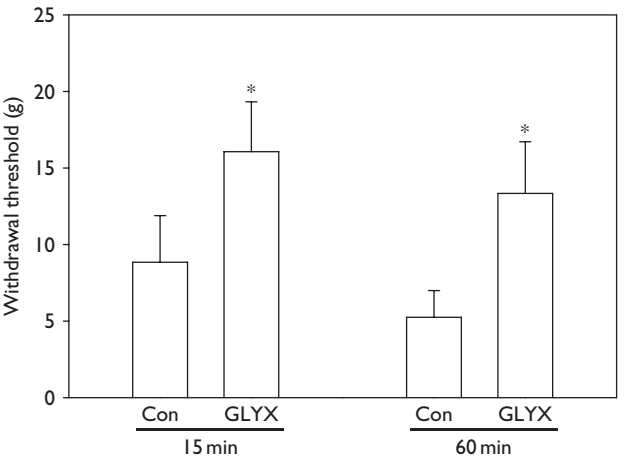

Fig. 2 Antinociceptive actions of GLYX-13 in the chronic constriction injury model of neuropathic pain. After development of peripheral neuropathy (12–13 days), GLYX-13 (5 mg/kg, intravenously) was administered to rats and mechanical allodynia evaluated with Von Frey fibers at 15 and 60 min after dosing. N=12–15 per group; Mean ± SEM; *, $P<0.05$ (The Von Frey data were logarithmically transformed and statistical comparisons made between treatments with an one-way analysis of variance followed by the Dunnett's *t*-test).

Unlike the study described in Fig. 1, these studies were performed intravenously. GLYX-13 was antinociceptive at both time points with no evidence of ataxia (Fig. 2). Ataxia was further evaluated utilizing a rotor-rod apparatus and GLYX-13 was not found to induce ataxia at 30 min after 5, 50, or 500 mg/kg intravenous administration. No data have been collected since past the 60 min time point. Mechanical allodynia is modulated by central rather than peripheral mechanisms [9]. GLYX-13 readily crosses the blood–brain barrier in an active form [6].

Discussion

GLYX-13 demonstrated significant antinociceptive activity in the rat formalin model of tonic pain and in the rat constriction nerve injury model of neuropathic pain at doses not inducing ataxia. This is in contrast to competitive receptor antagonists, which have been shown to alleviate neuropathic pain in preclinical studies, but were ineffective in clinical trials [e.g. 3-(2-carboxypoperazin-4-yl)propyl-1-phosphonic acid] [10]. Second generation competitive receptor antagonists, such as perzinfotel, have been shown to alleviate inflammatory neuropathic pain, but no clinical trial data are available [11]. Noncompetitive ion channel blockers have not proved to be of therapeutic value, as they typically show unacceptable psychotomimetic side effects (e.g. MK-801) [12]. GlycineB binding site antagonists do not show psychotomimetic side effects, but typically induce ataxia and sedation along with being poor 'blood–brain barrier crossers' [12]. Recently developed NR2B-specific noncompetitive receptor antagonists, such as traxoprodil [13], have made it to the clinic, but side effects such as dizziness and depression have also been reported. Finally, gabapentin (Neurontin) also has been reported to cause sedation, ataxia, and dizziness [14].

GLYX-13 was compared with gabapentin only in the formalin test because gabapentin (Neurotin) is widely used clinically and likely has mechanims of action that in part are

NMDA receptor-associated. The formalin assay for mechanical allodynia data were deemed adequate to make the comparison.

Conclusion

The results reported here with GLYX-13 show that NMDA receptor glycine-site partial agonists may be excellent therapeutic candidates for the treatment of some forms of neuropathic pain.

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