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Neuroimmunophilin Ligands Protect Cavernous Nerves after Crush Injury in the Rat: New Experimental Paradigms

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Abstract

Objectives—We investigated the effects of the orally bioavailable non-immunosuppressive immunophilin ligand GPI 1046 (GPI) on erectile function and cavernous nerve (CN) histology following unilateral or bilateral crush injury (UCI, BCI, respectively) of the CNs.

Methods—Adult male Sprague-Dawley rats were administered GPI 15 mg/kg intraperitoneally (ip) or 30 mg/kg orally (po), FK506 1 mg/kg, ip, or vehicle controls for each route of administration just prior to UCI or BCI and daily up to 7 d following injury. At day 1 or 7 of treatment, erectile function induced by CN electrical stimulation was measured, and electron microscopic analysis of the injured CN was performed.

Results—Intraperitoneal administration of GPI to rats with injured CN protected erectile function, in a fashion similar to the prototypic immunophilin ligand FK506, compared with vehicle-treated animals (93% ± 9% vs. 70% ± 5% vs. 45% ± 1%, $p < 0.01$, respectively). Oral administration of GPI elicited the same level of significant protection from CN injury. GPI administered PO at 30 mg/kg/d, dosing either once daily or four times daily with 7.5 mg/kg, provided nearly complete protection of erectile function. In a more severe BCI model, PO administration of GPI maintained erectile function at 24 h after CN injury. Ultrastructural analysis of injured CNs indicated that GPI administered at the time of CN injury prevents degeneration of about 83% of the unmyelinated axons at 7 d after CN injury.

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Take-home message

This study demonstrates that oral or intraperitoneal administration of GPI-1046, a non-immunosuppressive immunophilin ligand, recovers erectile function in rat models of cavernous nerve injury, thus providing a potential therapeutic tool for the treatment of erectile dysfunction related to nerve injury.

Disclosure

Conflict of interest: Under a licensing agreement between Guilford Pharmaceuticals and the Johns Hopkins University, A.L. Burnett is entitled to a share of royalty received by the university on sales of products described in this article. The university owns Guilford Pharmaceuticals stock, which is subjected to certain restrictions under university policy. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

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Conclusions—The orally administered immunophilin ligand GPI neuroprotects CNs and maintains erectile function in rats under various conditions of CN crush injury.

Keywords

Cavernous nerve injury; Erectile dysfunction; Immunophilin ligands

1. Introduction

Erectile dysfunction continues to be a significant problem for many men following radical prostatectomy surgery, even with the advent of nerve-sparing surgical techniques [1]. Although phosphodiesterase (type) 5 (PDE5) inhibitors, such as sildenafil, have proven to be beneficial to many men in improving the ability to have intercourse [2], there is still a necessity to prevent damage to the pelvic nerves that mediate erection. In recent studies, a number of researchers have focused on drugs that may minimize damage or accelerate erection recovery following radical prostatectomy [3–5]. The rat model of cavernous nerve (CN) crush injury relating to penile erection has been developed for use by researchers to mimic the damage and degeneration associated with nerve-sparing radical prostatectomy [6–8]. This model permits the investigation of neuroprotective agents for promoting nerve regeneration and functional erection recovery.

There have been a number of studies demonstrating neuroprotective and neuroregenerative efficacy of neuroimmunophilin ligands (NILs) [9]. In summary, *in vitro* assays have shown that NILs protected neuronal cells against N-methyl-D-aspartate toxicity, hydrogen peroxide toxicity, MPP⁺ and 6-hydroxydopamine (6-OHDA), excitotoxic lesions, and ddC and neuroblastoma cells. NILs have also provided protection or regenerative effects in intact animals as well. These studies include accelerated recovery in sciatic nerve crush, protective and regenerative effects in MPTP (1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine) mice, protection and regeneration in 6-OHDA lesioned rats, protection of motor neurons, and delay of disease onset in amyotrophic lateral sclerosis (ALS) transgenic mice, protection against focal ischemia, and protection against axonal damage following traumatic brain injury.

FK506, the prototype immunophilin ligand, protects CN function in a partial injury rat experimental model, which mimics damage following nerve-sparing radical prostatectomy clinically [6]. FK506 is also highly effective in maintaining erectile function in a more severely injured rat model involving focal transection of the CN [10]. In our current studies, we wished to evaluate whether the non-immunosuppressive neuroimmunophilin ligand GPI-1046 (GPI) protects erectile function following unilateral CN crush injury (UCI). In addition, we probed whether GPI preserves axonal integrity following CN crush injury. We also wished to evaluate the efficacy of GPI in an oral formulation using this CN crush injury model. Finally, we addressed the efficacy of GPI in a more severe injury paradigm consisting of bilateral CN crush injury (BCI).

2. Methods

Male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) aged 2–3 mo and weighing 250–350 g were used for these experiments. All rats were given free access to rodent chow and water *ad libitum*, and housed in groups of two to three animals with 12-h light/dark cycle. All experiments were done in accordance with Guilford Pharmaceutical Inc (Baltimore, MD, USA) Animal Care and Use Committee guidelines.

2.1. Experimental design

Each experiment consisted of at least three groups of age-matched animals: sham-operated group; UCI/BCI group treated with GPI (15 mg/kg/d, intraperitoneal [ip] or 30 mg/kg/d, orally [po]; Guilford Pharmaceuticals Inc) or FK506 (1 mg/kg/d, ip; Guilford Pharmaceuticals Inc); and vehicle-treated UCI/BCI group. Oral administration of GPI was by gavage once daily (30 mg/kg) or four times daily (7.5 mg/kg) for 1 or 7 d beginning on the day of the CN injury. The vehicles used (10% v/v in normal saline) for intraperitoneal and oral administration were Intralipid and Cremaphor EL (both from Sigma-Aldrich, St Louis, MO, USA), respectively.

2.2. Cavernous nerve crush injury

Rats were anesthetized with an intraperitoneal injection of ketamine and xylazine (60 mg/kg, 8 mg/kg, respectively; Penn Veterinary Supplies Inc, Lancaster, PA, USA). Cavernous nerves were isolated and crushed as described previously [6]. Specifically for UCI, the right CN was crushed with two 30-s applications of Dumont #5 (Fine Science Tools, Foster City, CA, USA) jeweler's forceps at about 1 mm below the major pelvic ganglion (MPG). For BCI, both the right and left CNs were isolated and crushed as described above. For sham animals, CNs were only identified and exposed. Animals were placed under an infrared heat lamp to maintain body temperature until fully recovered. Drugs or vehicle were administered 30 min prior to the crush surgery.

2.3. Intracavernosal pressure measurement

After 1- or 7-d treatments, intracavernosal pressure (ICP) responses induced by CN electrical stimulation were measured as described previously [6,10]. Briefly, under anesthesia, a bipolar stainless steel electrode was placed around the CN at 1–2 mm below the crush site, and ICP was monitored and recorded via a cannula inserted into the right crura. CN was stimulated (4 volts, 16 Herz, 5 ms) for 1 min for two to three times while allowing 15-min intervals between stimulations.

2.4. Electron microscopy

Animals were perfused with 4% paraformaldehyde. Right and left dorsolateral prostate tissue along with the MPG and associated CN were removed en bloc. Tissues were postfixed with paraformaldehyde (4%)-glutaraldehyde (3%) (Electron Microscopy Sciences, Ft Washington, PA, USA) at 4 °C for 3 d. The CNs were dissected out and placed in phosphate buffer until processed for electron microscopy (EM) by using standard methods as described previously [11,12]. Quantification of degenerating axons was conducted as described [11,12] by a person blind to the treatment protocol.

2.5. Data analysis

ICP responses were expressed as maximum ICP (mICP) or as %ICP of the uninjured CN stimulation for UCI. Each treatment group of animals was presented as a mean $\pm$ SE. One way analysis of variance (ANOVA) was used to compare results across treatment groups, with post hoc analysis (Newman Keuls) for multiple pair-wise comparisons. Statistical significance was defined at the $p < 0.05$ level.

3. Results

3.1. Crush injury model

In this model of crush injury, electrical stimulation of the crushed nerve (right CN) results in decreased mICP (Fig. 1A). With electrical stimulation of the uninjured contralateral nerve (left CN) there was a rapid rise in ICP, which was maintained for the duration of the stimulation. The right CN showed reduced average mICP with stimulation (Fig. 1B). Assessed at 24 h after

injury, mICP in rats treated with vehicle demonstrated about 50–60% reduction compared with sham injured rats. Not only was the amplitude of the ICP reduced, but also the rate of the pressure increase (change of ICP over time) was significantly reduced in right CN stimulation compared with left CN stimulation. The rate of ICP increase induced by CN stimulation in vehicle-treated rats decreased from 2.47 ± 0.4 to 1.2 ± 0.3 mm Hg/s from the uninjured to injured CN ($n = 7$, $p < 0.05$).

3.1.1. GPI and FK506 maintain ICP following intraperitoneal injection—In rats treated with GPI at 15 mg/kg, ip, 30 min prior to nerve crush, the mICP response to electrical stimulation of right CN nearly doubled compared to that of the vehicle controls (43 ± 2 vs. 23.6 ± 12 mm Hg, $p < 0.01$, Fig. 1B). In GPI-treated rats, the rate of the pressure increase and mICP to right CN stimulation were nearly identical to the left CN stimulation (Fig. 1A). The rate of ICP increase upon nerve stimulation in GPI-treated rats decreased only slightly (left CN, 2.43 ± 0.3 ; right CN, 2.1 ± 0.2 mm Hg/sec; $n = 6$, $p > 0.05$). The delay in the increase in ICP evoked by right CN stimulation in rats treated with the vehicle was not observed by stimulation of right CN in rats pretreated with GPI. The vehicle-treated rats displayed similar results to rats treated with normal saline vehicle, with no statistical difference. Both GPI and FK506 similarly maintained mICP levels following CN crush injury (Fig. 2). The mICP levels evoked by electrical stimulation of the crushed right CNs were normalized to the mICP levels measured on the intact left CNs. Thus, each animal served as its own control, reducing the variability of CN crush injury. FK506 treatment (1 mg/kg, ip) significantly protected against loss of evoked ICP compared with vehicle-treated rats, with mICP expressed as percent of the contralateral intact nerve ($70\% \pm 5$ vs. $45\% \pm 11$; $n = 6$, $p < 0.05$; Fig. 2). Furthermore, GPI treatment (15 mg/kg, ip) significantly prevented the reduction in ICP levels to an even greater extent than 1 mg/kg FK506 treatment. Similarly, GPI treatment showed improvement over vehicle in the UCI model when administered intraperitoneally for 3 d as well (data not shown).

3.1.2. Oral treatment with GPI maintains erectile function—Previous studies of GPI used in other nerve lesion paradigms have shown that, following oral administration of the compound, increased doses, nearly twice the intraperitoneal or subcutaneous dose are necessary to provide efficacy [13]. Thus, we utilized GPI at a dose of 30 mg/kg to assess the oral efficacy of the compound. When dosed orally, GPI protected against loss of mICP response induced by electrical stimulation at 24 h after injury (Fig. 3). GPI-treated animals showed significant improvement over the vehicle controls. The results of mICP measurements in rats treated with vehicle were similar to those of saline vehicle-treated rats (data not shown).

To extend these results, we evaluated the protective efficacy of GPI at 7 d after CN crush injury. We also found similar protective effects when GPI was given orally as a single daily dose at 30 mg/kg for 7 d (Fig. 4A). Rats with crushed CN displayed about 40% loss of mICP at 7 d after injury. Animals treated daily with GPI 30 mg/kg, po, for 7 d demonstrated nearly 100% of the contralateral ICP after 7 d (Fig. 4A). Since GPI has a short half-life in plasma following administration to animals [14], we administered multiple daily doses of the compound to evaluate biologic efficacy compared with those results observed following once-daily dosing with the compound. We tested whether the 30 mg/kg dose of GPI in four applications of 7.5 mg/kg, po, per day is equivalent to this once-daily 30 mg/kg dose for 7 d (Fig. 4B). Similar levels of protection were assessed by maintaining ICP levels to 90–100% of the contralateral nerves, whether the 30 mg/kg dose was given as a single oral dose or four smaller treatments totaling 30 mg/kg.

3.2. GPI protects ICP levels following BCI

We wished to assess the protective efficacy of GPI in a more severe injury model of erectile dysfunction. One such model, BCI, is produced by crushing both the right and left CNs. In this

model, we found about a 50–60% reduction of mICP induced by CN electrical stimulation in both CNs, measured at 24 h after crush injury. GPI, administered at 30 mg/kg, po, 30 min prior to BCI, provided significant protection to these nerves at 24 h (Fig. 5). Mean mICPs were 67 ± 4.0 mm Hg in non-CN-injured rats, 33 ± 2.8 mm Hg in vehicle-treated BCI rats, and 57 ± 5.2 mm Hg in GPI-treated BCI rats. These mICP values evoked from BCI/vehicle-treated rats were significantly reduced ($p < 0.001$) from ICP elicited from both BCI/GPI-treated and sham/vehicle-treated animals.

3.3. GPI treatment protects CN axons from damage following crush injury

We examined the CN distal to the partial crush site at the ultrastructural level to determine the morphologic substrate of the physiologic benefit seen with GPI (Fig. 6). Using an unbiased sampling method [6,15] we examined the number of remaining healthy axons in the distal CNs 7 d after the crush injury. Crush injury to the CN caused degeneration of about 50% of the unmyelinated axons distal to the crush site [6] (Fig. 6A). In contrast, administration of GPI at 15 mg/kg, ip, at the time of crush injury prevented degeneration of about 83% of the unmyelinated axons (Fig. 6A).

4. Discussion

Previous studies have demonstrated that FK506 and GPI protect erectile function and maintain peripheral nerve axon integrity following CN crush [6,10]. FK506 treatment demonstrates significant protection of CNs from crush injury [6]. In fact, treatment with FK506 maintains erectile function, as assessed by mICP responses, and axonal viability and morphology for up to 7 d after CN crush injury in rats. In the most recent study [10], we found that FK506 and GPI protect erectile function in a focal CN transection model of nerve injury-associated erectile dysfunction. Following unilateral and bilateral transection of the CN, both FK506 and GPI treatments promote significant erection recovery in treated animals. After 28 d of continuous treatment in rats with bilaterally transected CN, GPI-treated rats demonstrate significant erection recovery. In our current study, we found that the non-immunosuppressive neuroimmunophilin ligand GPI also maintained ICP levels and preserves CN axonal viability following CN crush. GPI displayed comparable protective efficacy to FK506 in our CN crush models, as well as similar protective effects on unmyelinated axons in the crushed CN nerve. Furthermore, we now have found significant protective efficacy of GPI in this CN crush model up to 7 d after injury with the use of the oral formulation of the compound. Finally, in a more severe model of injury involving bilateral CN crush injury, oral GPI treatment maintained erectile function in these animals.

Several different animal models of traumatic injury to the CN have been used to study the neuroprotective potential of immunophilin ligands. The CN crush model was developed to mimic the contusion or mechanical damage resulting from nerve-sparing radical prostatectomy surgery. The CN transection model offered an approach to study a more severe injury and also evaluate regenerative effects of these drugs [10]. In the transection model, the ICP loss is significantly greater than that in the CN crush model, typically approaching 75–80% reduction compared with sham-operated controls. FK506 treatment (5 mg/kg, subcutaneous [sc]) of rats with transected nerves elicited a significant recovery of ICP levels following a 5-d course of treatment [10]. Results of treatment with the non-immunosuppressive GPI in this model also led to increased ICP levels, although to lower levels than were found with this highly dosed FK506 treatment regimen. Chronic administration of GPI in this model was significantly more efficacious in restoring erectile function [10].

A high-affinity binding target for FK506 and GPI, FK506-binding protein (FKBP)-12, has been localized to intact and injured rat penile nerves [16]. There is also evidence to show that localization of FKBP-12 correlates with neuronal nitric oxide synthase (nNOS), an enzyme

which neuronally forms nitric oxide, the principal mediator of penile erection. Peripheral nerves are known for their regenerative characteristics following injury or structural damage [17]. This regeneration may occur at different rates, and upregulated nNOS expression appears to be essential for neuronal regeneration to occur [18,19]. The MPG is thought to be the major source of NOS-containing nerve fibers supplying the erectile tissue of the penis, and nNOS is involved in the relaxation of the smooth muscle of the corpora cavernosa. Upregulated NOS expression may be associated with the regenerative process of the CN following injury. Neuronal and endothelial isoforms of NOS and their messenger RNA in the penis and MPG of adult male rats have also been evaluated by Western and Northern blots. nNOS was detected in abundance in the MPG and within the crura of the penis, while eNOS was found to be more abundant in the penile and pelvic parts of the urethra, with moderate levels in the penile shaft, crura, neurovascular bundle, and MPG [20,21].

The protective mechanism of action of GPI and FK506 remains unclear. Treatment of cells in culture and intact animals with these compounds has demonstrated heightened levels of the antioxidant glutathione [22–24]. The level of reactive oxygen species in cells and tissues is also reduced by treatment with these compounds [22,23]. These compounds potentially affect steroid receptor complex activities in mediating their neurotrophic actions [25]. Potential actions on intracellular calcium release and homeostasis have also been proposed [26]. Recent evidence has indicated a role of these compounds in stimulating expression of the heat shock protein 70 [27]. Additional data has been published in which motor neurons are protected from excitotoxic glutamate lesions by low nanomolar concentrations of GPI [28]. Finally, Fischer and colleagues [29] have discovered that GPI may target a different FKBP, namely FKBP38 along with calcium/calmodulin to elicit their neuroprotective effects.

On the basis of protective effects observed in CN crush models and the more severe CN transection models, immunophilin ligands may provide therapeutic utility in radical prostatectomy surgeries. Providing trophic support with a neuroimmunophilin ligand would promote axonal integrity and neuronal viability during this traumatic event. Promoting neuronal survival would help maintain erectile function and may serve an adjunctive role with PDE5 inhibitors to facilitate erectile function. The use of NILs therapeutically will be supported to an even greater extent when these data have been replicated and validated by independent investigators. In fact, clinical trials have commenced using immunophilin ligand therapy to promote recovery of erectile function following bilateral nerve-sparing radical prostatectomy.

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References

1. Burnett AL. Erectile dysfunction following radical prostatectomy. *JAMA* 2005;293:2648–53. [PubMed: 15928287]
2. Montorsi F, McCullough A. Efficacy of sildenafil citrate in men with erectile dysfunction following radical prostatectomy: a systematic review of clinical data. *J Sex Med* 2005;2:658–67. [PubMed: 16422824]
3. Carrier S, Zvara P, Nunes L, et al. Regeneration of nitric oxide synthase-containing nerves after cavernous nerve neurotomy in the rat. *J Urol* 1995;153:1722–7. [PubMed: 7536273]

4. Bochinski D, Lin GT, Nunes L, et al. The effect of neural embryonic stem cell therapy in a rat model of cavernosal nerve injury. *BJU Int* 2004;94:904–9. [PubMed: 15476533]
5. Chen KC, Minor TX, Rahman NU, et al. The additive erectile recovery effect of brain-derived neurotrophic factor combined with vascular endothelial growth factor in a rat model of neurogenic impotence. *BJU Int* 2005;95:1077–80. [PubMed: 15839936]
6. Sezen SF, Hoke A, Burnett AL, Snyder SH. Immunophilin ligand FK506 is neuroprotective for penile innervation. *Nat Med* 2001;7:1073–4. [PubMed: 11590407]
7. Kendirci M, Zsengeller Z, Bivalacqua TJ, et al. Poly(adenosine diphosphate-ribose) polymerase inhibition preserves erectile function in rats after cavernous nerve injury. *J Urol* 2005;174:2054–9. [PubMed: 16217393]
8. Hsieh PS, Bochinski DJ, Lin GT, et al. The effect of vascular endothelial growth factor and brain-derived neurotrophic factor on cavernosal nerve regeneration in a nerve-crush rat model. *BJU Int* 2003;92:470–5. [PubMed: 12930443]
9. Poulter MO, Payne KB, Steiner JP. Neuroimmunophilins: a novel drug therapy for the reversal of neurodegenerative disease? *Neuroscience* 2004;128:1–6. [PubMed: 15450348]
10. Burnett AL, Becker RE. Immunophilin ligands promote penile neurogenesis and erection recovery after cavernous nerve injury. *J Urol* 2004;171:495–500. [PubMed: 14665962]
11. Brostoff SW, Levit S, Powers JM. Induction of experimental allergic neuritis with a peptide from myelin P2 basic protein. *Nature* 1977;268:752–3. [PubMed: 70758]
12. Larsen JO. Stereology of nerve cross sections. *J Neurosci Methods* 1998;85:107–18. [PubMed: 9874147]
13. Steiner JP, Hamilton GS, Ross DT, et al. Neurotrophic immunophilin ligands stimulate structural and functional recovery in neurodegenerative animal models. *Proc Natl Acad Sci U S A* 1997;94:2019–24.
14. Harper S, Bilsland J, Young L, et al. Analysis of the neurotrophic effects of GPI-1046 on neuron survival and regeneration in culture and in vivo. *Neuroscience* 1999;88:257–67. [PubMed: 10051205]
15. Hoke A, Ho T, Crawford TO, et al. Glial cell line-derived neurotrophic factor alters axon schwann cell units and promotes myelination in unmyelinated nerve fibers. *J Neurosci* 2003;23:561–7. [PubMed: 12533616]
16. Sezen SF, Blackshaw S, Steiner JP, Burnett AL. FK506 binding protein 12 is expressed in rat penile innervation and upregulated after cavernous nerve injury. *Int J Impot Res* 2002;14:506–12. [PubMed: 12494287]
17. Ide C. Peripheral nerve regeneration. *Neurosci Res* 1996;25:101–21. [PubMed: 8829147]
18. Keilhoff G, Fansa H, Wolf G. Neuronal nos deficiency promotes apoptotic cell death of spinal cord neurons after peripheral nerve transection. *Nitric Oxide* 2004;10:101–11. [PubMed: 15135363]
19. Thippeswamy T, Jain RK, Mumtaz N, Morris R. Inhibition of neuronal nitric oxide synthase results in neurodegenerative changes in the axotomised dorsal root ganglion neurons: Evidence for a neuroprotective role of nitric oxide in vivo. *Neurosci Res* 2001;40:37–44. [PubMed: 11311403]
20. Podlasek CA, Gonzalez CM, Zelner DJ, et al. Analysis of NOS isoform changes in a post radical prostatectomy model of erectile dysfunction. *Int J Impot Res* 2001;13 Suppl 5:S1–15. [PubMed: 11781741]
21. Gonzalez CM, Brannigan RE, Bervig T, et al. Protein and gene expression of nitric oxide synthase isoforms I and III in the rat penile shaft. *J Androl* 2001;22:54–61. [PubMed: 11191088]
22. Tanaka, K-i; Fujita, N.; Higashi, Y.; Ogawa, N. Neuroprotective and antioxidant properties of fkb-binding immunophilin ligands are independent on the FKBP-12 pathway in human cells. *Neurosc Lett* 2002;330:147–50.
23. Tanaka, K-i; Yoshioka, M.; Miyazaki, I.; Fujita, N.; Ogawa, N. GPI1046 prevents dopaminergic dysfunction by activating glutathione system in the mouse striatum. *Neurosci Lett* 2002;321:45–8. [PubMed: 11872253]
24. Tanaka, K-i; Fujita, N.; Yoshioka, M.; Ogawa, N. Immunosuppressive and non-immunosuppressive immunophilin ligands improve H2O2-induced cell damage by increasing glutathione levels in NG108-15 cells. *Brain Res* 2001;889:225–8. [PubMed: 11166708]

25. Gold BG, Villafranca JE. Neuroimmunophilin ligands: The development of novel neuroregenerative/neuroprotective compounds. *Curr Top Med Chem* 2003;3:1368–75. [PubMed: 12871168]
26. Lehnart SE, Huang F, Marx SO, Marks AR. Immunophilins and coupled gating of ryanodine receptors. *Curr Top Med Chem* 2003;3:1383–91. [PubMed: 12871170]
27. Klettner A, Herdegen T. The immunophilin-ligands FK506 and V-10,367 mediate neuroprotection by the heat shock response. *Br J Pharmacol* 2003;138:1004–12. [PubMed: 12642403]
28. Ganel R, Ho T, Maragakis NJ, et al. Selective up-regulation of the glial Na⁺-dependent glutamate transporter GLT1 by a neuroimmunophilin ligand results in neuroprotection. *Neurobiol Dis* 2006;21:556–67. [PubMed: 16274998]
29. Edlich F, Weiwad M, Erdmann F, et al. Bcl-2 regulator FKBP-38 is activated by ca²⁺/calmodulin. *EMBO J* 2005;24:2688–99. [PubMed: 15990872]

Fig. 1.

ICP is maintained in UCI following intraperitoneal treatment of GPI. The right CN was crushed as described in methods. Both vehicle and GPI (15 mg/kg, ip) treatment preceded the nerve crush by 30 min. Twenty-four hours later, ICP was measured during a 60-s electrical stimulation of the left CN (control) and the right CN (crush). (A). The ICP responses were recorded as a function of time and depicted for both the GPI-treated (left panel) and the vehicle-treated (right panel) rats. (B). Comparison of the maximum ICP response data from graphs in A. Statistical difference defined as * $p < 0.01$, $n = 6$ per group. CN = cavernous nerve; GPI = GPI-1046; ICP = intracavernosal pressure; ip = intraperitoneal; UCI = unilateral crush injury.

Fig. 2. Intraperitoneal administration of FK506 and GPI maintains ICP responses in rats with UCI. Rats were treated with vehicle, GPI (15 mg/kg), or FK506 (1 mg/kg) for 30 min prior to UCI. Twenty-four hours later, ICP was measured during a 60-s electrical stimulation of the left CN (control) and the right CN (crush). The ICP response was expressed as %ICP of the uninjured CN stimulation for UCI. Statistical difference defined as * $p < 0.05$, ** $p < 0.01$, $n = 6$ per group. See Figure 1 for definitions of abbreviations.

Fig. 3.

Oral administration of GPI maintains ICP responses in rats with UCI. Both the vehicle and GPI (30 mg/kg) treatment preceded the nerve crush by 30 min. Twenty-four hours later, ICP was measured during a 60-s electrical stimulation of the left CN (control) and the right CN (crush). Data were expressed as mean $\pm$ SE of maximum ICP. Statistical difference defined as * $p < 0.05$, $n = 8$ per group. See Figure 1 for definitions of abbreviations.

Fig. 4.

Oral administration of GPI maintains ICP responses in rats 7 d after UCI. Rats were treated for 7 d with vehicle or GPI starting 30 min prior to UCI. (A) Vehicle or GPI (30 mg/kg/d). (B) Vehicle or GPI (7.5 mg/kg, po). Seven days after UCI, ICP was measured during a 60-s electrical stimulation of the left CN (control) and the right CN (crush). The ICP response was expressed as %ICP of the uninjured CN stimulation. Statistical difference defined as ** $p < 0.01$, $n = 8/\text{group}$. See Figure 1 for definitions of abbreviations.

Fig. 5.

Oral administration of GPI maintains ICP responses in rats with BCI. Rats were administered vehicle or GPI (30 mg/kg) 30 min prior to BCI. A group of sham rats were run in parallel for comparison. Twenty-four hours later, ICP was measured during a 60-s electrical stimulation of the left CN (control) and the right CN (crush). Data was expressed as mean $\pm$ SE of maximum ICP. Statistical difference defined as ** $p < 0.001$, $n = 8$ per group. BCI = bilateral crush injury; see Figure 1 for definitions of other abbreviations.

Fig. 6.

GPI treatment prevents degeneration of unmyelinated axons in the distal CN. Rats with UCI were treated with vehicle or GPI (15 mg/kg, ip), and the CNs were evaluated for axonal degeneration by EM (see Methods). (A) Quantitation of the right CN (crush) unmyelinated axon survival at 7 d after injury in vehicle- or GPI- treated animals ($N = 4$ per group, $p = 0.007$). Representative EMs of (B) left CN (control); (C) right CN (crush) with GPI treatment; (D) right CN (crush) with vehicle treatment. There are more degenerating unmyelinated axons in the vehicle-treated nerves (narrow arrows point to some of the degenerating axons; thick arrow points to a degenerating small myelinated axon). EM = electron microscopy; see Figure 1 for definitions of other abbreviations.