

Sympathetic Vasoconstrictor Antagonism and Vasodilatation Relieve Mechanical Allodynia in Rats With Chronic Postischemia Pain

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Abstract

Chronic pain that responds to antisympathetic treatments and α -adrenergic antagonists is clinically referred to as sympathetically maintained pain. Animal models of neuropathic pain have shown mixed results in terms of antinociceptive effectiveness of antisympathetic agents. The effectiveness of these agents have not been yet investigated in animal models of complex regional pain syndrome-type 1 (CRPS-I). In this study, we examined the effectiveness of antisympathetic agents and sympathetic vasoconstrictor antagonists, as well as agents that are vasodilators, in relieving mechanical allodynia in a recently developed animal model of CRPS-I (chronic postischemia pain or CPIP) produced by three hours of hind paw ischemia-reperfusion injury. Systemic guanethidine, phentolamine, clonidine, and prazosin are effective in reducing mechanical allodynia particularly at 2 days after reperfusion, and less so at 7 days after reperfusion. A nitric oxide donor vasodilator, SIN-1, also reduces mechanical allodynia more effectively at 2 days after reperfusion, but not at 7 days after reperfusion. These results suggest that the pain of CPIP, and possibly also CRPS-I, is relieved by reducing sympathetically mediated vasoconstriction, or enhancing vasodilatation.

Keywords

Complex regional pain syndrome; ischemia-reperfusion injury; neuropathic pain; allodynia; sympathetic block; adrenergic receptors

Complex regional pain syndrome-type I (CRPS-I) is a disorder that occurs after fracture, soft tissue or crush injury.¹⁶ Symptoms of CRPS-I include spontaneous burning (cutaneous) and aching (deep) pain, hyperalgesia, allodynia, and disorders of vasomotor and sudomotor regulation.^{27,57} CRPS-II is similar but also exhibits a clinically verified nerve injury.⁷⁴

Although controversial, sympathetic blocks are often reported to relieve CRPS pain.^{10,29,68} CRPS pain is also relieved with α -adrenergic antagonists such as phentolamine or phenoxybenzamine,^{48,52} agents that have been used as diagnostic tools for identifying so-called sympathetically maintained pain (SMP).^{3,76} Although clinical studies have not determined the specific mechanisms of SMP, CRPS-I patients display abnormal responses to sympathetic stimulation⁷⁵ and enhanced vasoconstrictive responses to norepinephrine (NE),^{1,4} as well as painful responses to intradermal NE.⁶⁹ Pain relief in CRPS patients usually follows warming of the affected limb,^{5,62,70} suggesting that vasodilatation may be important to pain relief by sympathetic blockade in CRPS.⁷⁰

Sympathectomy also relieves neuropathic pain in animals with spinal nerve ligation (SNL), chronic constriction injury of the sciatic nerve (CCI), and partial sciatic nerve ligation^{32,65} (PSNL) (however, see Ringkamp et al⁵⁴) but not cryoneurolysis or sural and tibial nerve transection.^{24,77} α -Adrenergic antagonists also relieve neuropathic pain in animal models^{26,34,41} (but see Park et al⁵¹; Ringkamp et al⁵⁵). Mechanisms that have been suggested for SMP in these models of CRPS-II include sympathetic efferent–primary afferent coupling,¹² possibly after sympathetic fiber sprouting,^{40,53} or de novo adrenergic sensitivity in damaged afferents,^{17,72} dorsal root ganglion cells,^{18,47} or afferent nociceptors.^{50,59} De novo adrenergic sensitivity may depend on upregulation of adrenoceptors on primary afferent nerve fibers^{7,13,79} or other indirect mechanisms such as tissue ischemia due to enhanced vasoconstrictive responsiveness to NE. Importantly, rats with CCI have impaired vasoconstriction to sympathetic stimulation and enhanced vasoconstriction to exogenous NE.^{36,37}

We recently introduced chronic postischemia pain (CPIP) as an animal model of CRPS-I produced after hind paw ischemia-reperfusion (I-R) injury.¹⁴ This animal model shows signs of I-R injury such as no-reflow and vascular abnormalities, as well as nociceptive and vascular hypersensitivity to norepinephrine.^{38,78} The pain-relieving effects of antisympathetic agents have not previously been tested in animal models of CRPS-I.^{21,22,71} In this report, we examine whether antisympathetic drugs, and agents that are vasodilators, are effective in reducing painful symptoms in this model. Thus, we test the effectiveness of sympathetic block with guanethidine, or systemic treatments with the α 1- and α 2-adrenergic antagonists prazosin and yohimbine, the α 2-adrenergic agonist clonidine, and a nitric oxide donor, in relieving mechanical allodynia in CPIP rats at 2 and 7 days after reperfusion.

Materials and Methods

Animals

Male Long-Evans hooded rats (275–325 g; Charles River, Quebec, Canada) were housed in groups of 3 to 4, with food and water available ad libitum, on a 12:12-hour light:dark cycle. All treatments and testing procedures were approved by the Animal Care Committee at McGill University, and conformed to the ethical guidelines of the Canadian Council on Animal Care and the International Association for the Study of Pain.

Animal Model of CRPS-I

Chronic postischemia pain was generated after exposure to prolonged hind paw ischemia and reperfusion as described in Coderre et al.¹⁴ Briefly, rats were anesthetized over a 3- to 4-hour period with a bolus (40 mg/kg, i.p.) and chronic intraperitoneal infusion of sodium pentobarbital for 2 hours (13 mg/h for the first hour, 6.5 mg/h for the second hour). After induction of anesthesia, a Nitrile 70 Durometer O-ring (O-Rings West, Seattle, WA) with a 7/32-inch internal diameter was placed around the rat's left hind limb just proximal to the ankle joint for 3 hours. We standardized the position of the O-ring to a point on the limb just proximal to the medial malleolus of the tibia. The termination of sodium pentobarbital anesthesia was timed so that rats recovered fully within 30 to 60 minutes after reperfusion, which occurred immediately after removal of the O-ring. Sham rats received exactly the same treatment, except that the O-ring was cut, so that it only loosely surrounded the ankle and did not occlude blood flow to the hind paw.

Mechanical Sensitivity Nociceptive Testing

Hind paw mechanical thresholds were assessed by measuring the withdrawal response to von Frey filament stimulation according to a modification of the up/down method described by Chaplan et al.¹¹ In brief, rats were placed in a plexiglas box ($21 \times 16 \times 27 \text{ cm}^3$) with a wire grid bottom through which the von Frey filaments (nylon monofilaments; Stoelting, Woodale, IL) were applied to the plantar surface of the hind paw. Filaments were applied in either ascending or descending strength as necessary to determine the filament closest to the threshold of response. Each filament was applied once for 10 seconds to the center of the paw between the pads, and a lower intensity hair followed each positive response, whereas a higher intensity hair followed each negative response (until 5 responses were recorded after a first change in response). The minimum stimulus intensity was 0.25 g and the maximum was 15 g. Based on the response pattern and the force of the final filament, the 50% response threshold (grams) was calculated. The resulting pattern of positive and negative responses was tabulated, and the 50% response threshold was interpolated using the formula $50\% \text{ threshold} = (10^{[x_f + k\delta]})/10,000$, where x_f = the value (in log units) of the final von Frey hair used; k = tabular value¹¹ for pattern of positive/negative responses; and δ = mean difference (in log units) between stimuli (here, 0.224). Hairs were from the standard Semmes-Weinstein series.⁶³

Drugs

Guanethidine, phentolamine, prazosin, yohimbine, and clonidine were all obtained from Sigma (Oakville, Ontario, Canada). 3-Morpholinylsydnoneimine chloride (SIN-1) was obtained from Tocris Bioscience (Ellisville, MO). All agents were diluted in a 0.9% saline vehicle.

Drug Administration Protocol

Mechanical sensitivity was tested before CPIP induction and before drug administration on day 2 or day 7 days after reperfusion (predrug) and at various times after drug administration. To avoid testing rats that did not exhibit allodynia, it was decided a priori that, for all groups, only rats with post-reperfusion (predrug) von Frey thresholds less than 6

g were used in the drug trials (accordingly, the mean and SEM for baseline von Frey thresholds only include rats that were subsequently given drug treatments).

For sympathetic block experiments, vehicle or guanethidine (30 mg/kg) was injected subcutaneously twice (separated by 24 hours) on either days 2 and 3 or days 7 and 8 after reperfusion. Mechanical allodynia was tested 4 hours after the second injection on day 3 (for days 2–3 guanethidine) or day 8 after reperfusion (for days 7–8 guanethidine). Separate groups of rats that received vehicle or guanethidine injections on days 2 to 3 were tested on days 3, 5, 9, and 14 after reperfusion. Previous studies have shown that even a single dose of systemic guanethidine is sufficient for long-term sympathetic blockade.^{33,45} For dose-effect studies with phentolamine (1–5 mg/kg), prazosin (1–5 mg/kg), yohimbine (1–5 mg/kg), clonidine (0.01–0.1 mg/kg), and SIN-1 (1–10 mg/kg), the drugs were administered intraperitoneally, and mechanical allodynia was tested 20 minutes later on either day 2 or 7 after reperfusion. Separate rats were tested on days 2 and 7. For time-course studies, rats were tested over a 3-hour period on day 2 after reperfusion with the highest dose of each drug (except guanethidine). All drug dosages were selected on the basis of previous studies examining the effects of these agents on nociception.^{26,34,44,51,55,64,67} Furthermore, all drug doses that were used were determined not to induce significant abnormalities in the rotorod test. The highest doses of phentolamine, prazosin, and yohimbine used produced a significant inhibition of NE-induced reductions in hind paw blood flow as measured by laser Doppler flowmetry (data not shown). All experiments were performed by using a randomized blocks design, and at the time of testing the experimenter was blind to the animal's treatment. Six to 7 rats were used per group.

Statistics

Group comparisons were analyzed using a 2-way repeated-measures ANOVA followed by Fisher's post hoc tests. Preinjury baseline von Frey thresholds are included in the figures for each drug trial but are not included in the statistical analyses.

Results

Approximately 70% of rats subjected to the CPIP procedure displayed mechanical allodynia (von Frey threshold below 6 g) and were used in the drug trials. There were no significant differences between groups in baseline paw-withdrawal thresholds or predrug trial thresholds at 2 days or 7 days after reperfusion.

Effect of Sympathetic Block on CPIP Mechanical Allodynia

Fig 1A shows von Frey thresholds of CPIP rats before and after guanethidine or vehicle treatment starting on day 2 after reperfusion. Two-way ANOVA reveals significant main effects of group ($F(1, 23) = 5.77, P < .05$) and time ($F(1, 23) = 4.85, P < .05$) and a significant group_time interaction ($F(1, 23) = 19.33, P < .01$). The vehicle injection did not produce a significant anti-allodynic effect ($P > .05$); however, guanethidine-treated rats displayed a significant increase in paw withdrawal threshold after the 2-day guanethidine treatment ($P < .01$). Continuing daily guanethidine treatment for up to 7 days did not result in any further reduction of mechanical allodynia (data not shown). Fig 1B shows von Frey thresholds of

CPIP rats before and after guanethidine or vehicle treatment starting on day 7 after reperfusion. Two-way ANOVA reveals non-significant main effects of group ($F(1, 23) = 0.05$, $P > .05$) and time ($F(1, 23) = 1.12$, $P > .05$) and a non-significant group X time interaction ($F(1, 23) = 0.12$, $P > .05$). This suggests that at this time point on day 8, the 2-day guanethidine treatment did not reduce mechanical allodynia. Fig 1C shows von Frey thresholds of CPIP rats before and for 14 days after vehicle or guanethidine treatment on days 2 and 3 after reperfusion. Two-way ANOVA reveals a significant effect of group ($F(1, 64) = 7.20$; $P < .05$) and time ($F(4, 64) = 2.81$; $P < .05$), and a non-significant group X time interaction ($F(4, 64) = 1.97$, $P > .05$). The guanethidine treatment resulted in significant increase of paw withdrawal threshold as compared with day 2 predrug baseline, at day 3 ($P < .01$), day 5 ($P < .05$), and day 14 ($P < .01$). The vehicle treatment did not result in any significant increase of paw withdrawal threshold at any of the time points tested.

Effect of the Mixed α_1/α_2 -Adrenergic Antagonist Phentolamine on CPIP Mechanical Allodynia

Fig 2A shows the von Frey thresholds of 2-day CPIP rats before and 20 minutes after treatment with vehicle or 1, 2.5, and 5 mg/kg phentolamine. Two-way ANOVA revealed a significant main effect of time (pre-post) ($F(1, 47) = 8.05$, $P < .05$), a non-significant main effect of dose ($F(3, 47) = 0.58$, $P < .05$), and a non-significant time X dose interaction ($F(3, 47) = 2.05$, $P > .05$). Neither the vehicle, 1 mg/kg phentolamine, nor 2.5 mg/kg phentolamine injection doses resulted in significant increases in paw withdrawal thresholds ($P > .05$). However, the time effect was significant because the 5 mg/kg phentolamine significantly increased in paw withdrawal threshold after the drug as compared with before the drug ($P < .01$). Fig 2B shows the von Frey thresholds of 7-day CPIP rats before and 20 minutes after treatment with vehicle or 1, 2.5, and 5 mg/kg phentolamine. Two-way ANOVA reveals non-significant main effects of time (pre-post) ($F(1, 47) = 1.36$, $P > .05$) and dose ($F(3, 47) = 0.52$, $P > .05$) as well as a non-significant dose X time interaction ($F(3, 47) = 0.43$, $P > .05$). Therefore, it appears that phentolamine is not effective at 7 days after reperfusion, and only the highest dose at 2 days after reperfusion is able to reduce mechanical allodynia in CPIP rats.

Effect of the α_1 -Adrenergic Antagonist Prazosin on CPIP Mechanical Allodynia

Fig 3A shows the von Frey thresholds of 2-day CPIP rats before and 20 minutes after treatment with vehicle or 1, 2.5, and 5 mg/kg prazosin. Two-way ANOVA reveals significant main effects of time (pre-post) ($F(1, 47) = 28.27$, $P < .0001$) and dose ($F(3, 47) = 3.90$, $P < .05$) and a significant time X dose interaction ($F(3, 47) = 4.59$, $P < .05$). The vehicle injection did not result in a significant increase in paw withdrawal threshold ($P > .05$). All 3 prazosin doses, 1, 2.5, and 5 mg/kg, resulted in significant increases in paw withdrawal thresholds ($P < 0.01$). Fig 3B shows the von Frey thresholds of 7-day CPIP rats before and 20 minutes after treatment with vehicle or 1, 2.5, and 5 mg/kg prazosin. Two-way ANOVA reveals a significant main effect of time (pre-post) ($F(1, 47) = 8.80$, $P < .01$), a non-significant main effect of dose ($F(3, 47) = 0.67$, $P > .05$), and a non-significant time X dose interaction ($F(3, 47) = 0.95$, $P > .05$). The vehicle injection did not result in a significant increase in paw withdrawal threshold ($P > .05$). The time effect was significant because the 2.5 mg/kg dose significantly increased the paw withdrawal thresholds after the drug as compared with

before the drug ($P > .05$). However, neither the 1 mg/kg nor the 5 mg/kg prazosin doses were able to significantly increase the paw withdrawal thresholds relative to predrug levels ($P < .05$). However, rats that received the 5 mg/kg dose of prazosin did have significantly higher von Frey thresholds than rats that received vehicle ($P < .05$). It appears that prazosin is able to reduce mechanical allodynia at both 2 and 7 days after reperfusion, and is more effective at 2 days after reperfusion in CPIP rats.

Effect of the α_2 -Adrenergic Antagonist Yohimbine on CPIP Mechanical Allodynia

Fig 4A shows the von Frey thresholds of 2-day CPIP rats before and 20 minutes after treatment with vehicle or 1, 2.5, and 5 mg/kg yohimbine. Two-way ANOVA shows non-significant main effects of time (pre-post) ($F(1, 47) = 0.61, P > .05$) and dose ($F(3, 47) = 0.01, P > .05$) as well as a Non-significant time X dose interaction ($F(3, 47) = 1.02, P > .05$). This suggests that none of these doses of yohimbine are able to relieve mechanical allodynia at 2 days after reperfusion. Fig 4B shows the von Frey thresholds of 7-day CPIP rats before and 20 minutes after treatment with vehicle or 1, 2.5, and 5 mg/kg yohimbine. Two-way ANOVA shows non-significant main effects of time (prepost) ($F(1, 47) = 0.56, P > .05$) and dose ($F(3, 47) = 0.66, P > .05$) as well as a non-significant time X dose interaction ($F(3, 47) = 0.19, P > .05$). Therefore, it appears that yohimbine is unable to reduce mechanical allodynia in CPIP rats either at 2 days or 7 days after reperfusion.

Effect of the α_2 -Adrenergic Agonist Clonidine on CPIP Mechanical Allodynia

Fig 5A shows the von Frey thresholds of 2-day CPIP rats before and 20 minutes after treatment with vehicle or 0.01, 0.025, and 0.1 mg/kg clonidine, as well as pretreatment with a 5 mg/kg yohimbine dose before a 0.1 mg/kg clonidine injection. Two-way ANOVA reveals a significant main effect of time (pre-post) ($F(1, 59) = 12.34, P < .01$), a non-significant main effect of dose ($F(4, 59) = 2.39, P > .05$), and a significant time X dose interaction ($F(4, 59) = 3.55, P < .05$). Neither the vehicle injection nor the 0.01 mg/kg clonidine dose resulted in a significant increase in paw withdrawal threshold ($P > .05$). Both the 0.025 mg/kg and the 0.1 mg/kg clonidine doses resulted in significant increases in paw withdrawal thresholds ($P < .05$ and $P < .01$, respectively). Further, pretreatment with yohimbine fully inhibited any increase in paw withdrawal threshold observed with the highest dose of clonidine ($P > .05$), confirming that the effects are α_2 -adrenergic receptor mediated. Fig 5B shows the von Frey thresholds of 7-day CPIP rats before and 20 minutes after treatment with vehicle or 0.01, 0.025, and 0.1 mg/kg clonidine. Two-way ANOVA shows a significant main effect of time (pre-post) ($F(1, 47) = 9.34, P < .01$), a non-significant main effect of dose ($F(3, 47) = 1.1, P > .05$), and a non-significant time X dose interaction ($F(3, 47) = 0.97, P > .05$). Neither the vehicle injection, the 0.01 mg/kg, nor the 0.025 mg/kg clonidine doses resulted in significant increases in paw withdrawal threshold ($P > .05$). The time effect was significant, however, since the 0.1 mg/kg clonidine dose significantly increased in paw withdrawal thresholds after the drug as compared with before the drug ($P < .05$). Therefore, it appears that clonidine is able to significantly reduce mechanical allodynia at 2 days, and lesser so at 7 days, after reperfusion.

Time Course of Anti-allodynia of Adrenergic Agents on CPIP

Fig 6 shows an anti-allodynia time course of the highest dose of the above drugs (except guanethidine) in 2-day CPIP rats at 20, 40, 60, 80, 120, and 180 minutes after drug injection. Two-way ANOVA revealed significant main effects of group ($F(4, 209) = 14.40, P < .0001$) and time ($F(6, 209) = 3.29, P < .0001$) as well as a significant group X time interaction ($F(24, 209) = 2.92, P < .0001$). None of the post-injection paw withdrawal thresholds in the vehicle group are significantly different from the pre-injection paw withdrawal threshold. For phentolamine, paw withdrawal thresholds are significantly increased compared with vehicle at the 60-minute time point ($P < .05$). For prazosin, paw withdrawal thresholds are significantly increased compared with vehicle at the 20-, 40-, 60-, 80- ($P < .01$), and 120-minute ($P < .05$) time points. For yohimbine, paw-withdrawal thresholds fail to differ significantly from vehicle at any time point. Finally, for clonidine, paw withdrawal thresholds are significantly increased compared with vehicle at the 20-, 40-, 60-, 80- ($P < .01$), and 120-minute ($P < .05$) time points. Therefore, phentolamine, prazosin, and clonidine, but not yohimbine, are able to relieve mechanical allodynia with peak anti-allodynia observed approximately 60 minutes after injection.

Effect of a Nitric Oxide Donor Vasodilator (SIN-1) on CPIP Mechanical Allodynia

Fig 7A shows the von Frey thresholds of 2-day CPIP rats before and 20 minutes after treatment with vehicle or 1, 3, and 10 mg/kg of SIN-1. Two-way ANOVA revealed a significant main effect of time ($F(1, 53) = 24.02, P < .0001$), a non-significant main effect of dose ($F(3, 53) = 2.54, P > .05$), and a significant time X dose interaction ($F(3, 53) = 4.87, P < .01$). The vehicle injection did not result in a significant increase in paw withdrawal threshold ($P > .05$). All 3 doses, 1, 2.5 ($P < .05$), and 10 mg/kg ($P < .01$), resulted in significant increases in paw withdrawal thresholds. Fig 7B shows the von Frey thresholds of 7-day CPIP rats before and 20 minutes after treatment with vehicle or 1, 3, and 10 mg/kg of SIN-1. Two-way ANOVA revealed non-significant main effects of time ($F(1, 47) = 2.75, P > .05$) and dose ($F(3, 47) = 1.05, P > .05$) as well as a non-significant time X dose interaction ($F(3, 47) = 2.89, P > .05$). Therefore, SIN-1 is able to reduce mechanical allodynia at 2 days but not at 7 days after reperfusion in CPIP rats.

Discussion

A contribution of the sympathetic system to pain mechanisms has been hypothesized in a variety of animal models of nerve injury and inflammation. Clinically, it has been suggested that it could be useful to consider subtypes of CRPS-I classified as SMP and sympathetically independent pain (SIP).^{9,56} However, the effectiveness of sympathectomy and sympathetic blockade as standardized treatment for CRPS has been questioned.^{35,42} In animal models, sympathectomy has been shown to partially reduce mechanical allodynia after chronic constriction injury, sciatic nerve ligation, and spinal nerve ligation.^{32,33,43,65} In CPIP rats, we found that sympathetic block with guanethidine partially reduced mechanical allodynia for up to 14 days when administered on days 2 and 3 after reperfusion; however, this effect was not significant when guanethidine was administered on days 7 and 8 after reperfusion. This is consistent with the clinical literature in which sympathetic blocks or

sympathectomy may only be partially effective for the overall CRPS population, but also that they are particularly more effective early in the disease.^{8,66}

Systemic administration of the nonspecific α -adrenergic antagonist phentolamine also partially reduces mechanical allodynia in CPIP rats at 2 days after reperfusion but, again, not significantly at 7 days after reperfusion. Along with sympathetic blocks, the response to phentolamine has been used clinically to identify SMP.⁵² Our results also resemble those in studies using rat spinal nerve ligation, in which both sympathectomy and systemic phentolamine reduce pain behaviors,^{33,79} but this has not been consistently demonstrated.^{54,55} It has been suggested that for CRPS patients, the response to phentolamine predicts response to sympathetic blocks⁷⁶ and regional guanethidine.³ CPIP rats are similar to CRPS patients because their allodynia is relieved both by phentolamine and guanethidine. Phenoxybenzamine, another nonselective α_1 -antagonist, is also effective in CRPS,⁴⁸ although we did not study it here.

We found that the α_1 -adrenergic antagonist prazosin, but not the α_2 -antagonist yohimbine, effectively reduces mechanical allodynia, with almost a complete reversal for the 5 mg/kg prazosin dose in 2-day CPIP rats. This strongly suggests that the α_1 -receptor contributes to the mechanical allodynia in CPIP. Lee et al⁴¹ found that α_1 -adrenergic antagonists, but not α_2 -adrenergic antagonists, also reduce mechanical allodynia in rats with segmental spinal injury. Phentolamine and α_1 - but not α_2 -adrenergic antagonists also reduce cold allodynia in rats with S1/S2 spinal nerve ligation.³⁴ Heat hyperalgesia in CCI rats is also reduced by α_1 -adrenergic antagonists.²⁶ Therefore, it appears that the CPIP model of CRPS -I displays similarities to animal models of CRPS-II, with each showing characteristics of SMP, and a critical role for α_1 -receptors in SMP. As for the clinical use of these agents in CRPS, there are no carefully controlled trials with selective α_1 -or α_2 -adrenergic antagonists.⁵⁷

The finding that yohimbine did not produce anti-allodynia in our model and has even been found to exacerbate cold allodynia in rats with spinal nerve ligation³⁴ suggests that antagonists of α_2 -adrenergic receptors may have detrimental effects rather than beneficial ones in these models. Since many α_2 -adrenergic receptors are autoreceptors mediating negative feedback on NE release from sympathetic postganglionic neurons (SPGNs),^{28,60} α_2 -antagonists are known to enhance SPGN NE release.³⁹ The enhanced NE release would increase vasoconstriction, which we expect would be detrimental in CPIP rats, which already have poor blood flow.³⁸ In a similar fashion, the action of phentolamine as an α_2 -adrenergic antagonist may also be reducing its antiallodynic actions at the α_1 -adrenergic receptor, providing a possible explanation for why phentolamine is less effective than prazosin in CPIP rats.

Like prazosin, systemic clonidine is particularly effective in CPIP rats at doses that do not produce defects on the rotorod. Studies have shown that systemic clonidine can produce analgesic effects in humans²³ and topical administration relieves SMP in CRPS patients.¹⁵ Systemic clonidine also partially relieves mechanical allodynia in rodents with CCI of the sciatic nerve,³⁰ and topical application has also been shown to be antinociceptive in animal studies.¹⁹ It is tempting to speculate that the analgesic effects of clonidine in CPIP rats are based on its known negative feedback effects on NE release from SPGNs.^{28,60} The α_2 -

agonist clonidine may also be particularly effective due to a combination of spinal analgesic and local vasodilatory effects.^{20,25} We have previously shown that the I-R injury associated with CPIP produces persistent tissue ischemia, indicated by reduced muscle perfusion,³⁸ as well as vasoconstrictor hyper-responsiveness, indicated by an enhanced reduction in hind paw blood flow after close arterial injection of NE.⁷⁸ The results here are consistent with a vasoactive role for NE from sympathetic efferents, in which activity at α_1 -receptors is pronociceptive due to vasoconstriction in already ischemic tissue. Blocking the α_1 -receptor with prazosin, therefore, results in vasodilatation and pain relief.

In the CPIP rats, we hypothesize that the I-R injury results in persistent tissue ischemia that contributes to the pain. Some of the known changes of I-R injury include vascular abnormalities such as persistent ischemia, dependent on either no-reflow due to capillary clogging,⁶ or arterial vasospasms due to sympathetic vasoconstrictor hyper-responsiveness and/or endothelial cell dysfunction. Indeed, after I-R injury there is both an upregulation and hyper-responsiveness of vascular adrenoceptors⁶¹ as well as a reduced production and vasodilatory function of nitric oxide.^{31,58}

If persistent tissue ischemia contributes to CPIP pain,³⁸ then CPIP pain should also be relieved by agents that enhance vasodilatation. In this study, we found that allodynia in CPIP rats was dose-dependently reduced by systemic administration of the nitric oxide donor SIN-1. We propose that by increasing the levels of nitric oxide, SIN-1 induces vasodilatation that relieves pain-producing vasospasms and ischemia in CPIP rats. Indeed, it has been shown that blood flow can be improved in ischemic tissue with exogenous NO.^{46,73} Further, our results show that anti-allodynic doses of SIN-1, as well as another NO donor (sodium nitroprusside), also attenuate NE-evoked pain in CPIP rats.⁷⁸ Therefore, it seems likely that NO-mediated vasodilation can reduce persistent tissue ischemia, and this may be a useful treatment for SMP.

In the CPIP rats, we find that antisympathetic and vasodilatory drugs are more effective at 2 days after reperfusion rather than 7 days after reperfusion. The temporal reduction in effectiveness may depend partly on a shift in the reliance of persistent ischemia on arterial vasospasms to no-reflow, a phenomenon that develops quickly after prolonged ischemia or repeated I-R injury.^{2,38,49} In CRPS-I patients, a slower development of no-reflow may also result in chronic tissue ischemia and pain that is more resistant to relief by sympathetic blockers.

In conclusion, CPIP rats show evidence of SMP (as defined clinically), since their mechanical allodynia is relieved by both sympathetic block and systemic phentolamine treatment. Furthermore, the responses to prazosin, clonidine, and the nitric oxide donor SIN-1 demonstrates that pain relief in CPIP rats can be produced by agents that decrease sympathetic vasoconstriction or enhance vasodilatation. We conclude that SMP mechanisms may involve exaggerated sympathetically mediated vascular contractility and persistent tissue ischemia.

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Perspective

The results of this study indicate that sympathetic block, or administration of α_1 -adrenergic antagonists, clonidine, or a nitric oxide donor, relieve allodynia in an animal model of CRPS-I. Thus, the pain of CRPS-I may depend on enhanced vasoconstrictor responsiveness, which may be relieved by blocking sympathetic efferent-dependent vasoconstriction, or by enhancing nitric oxide-dependent vasodilatation.

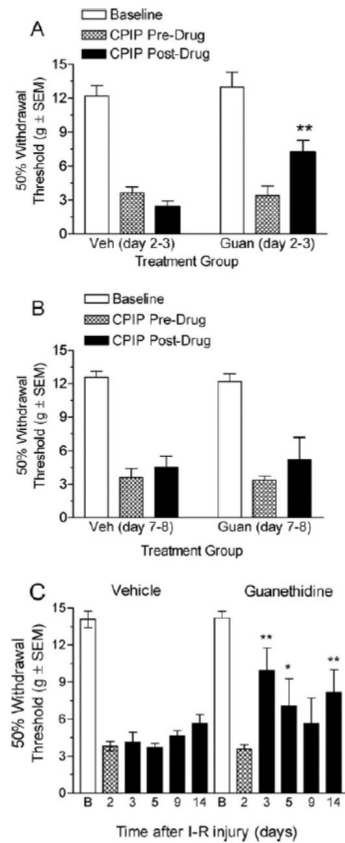


Figure 1.

A, Effect of sympathetic block with guanethidine on mechanical allodynia in chronic postischemia pain (CPIP) rats at 3 days after reperfusion. CPIP rats display a significant reduction in mechanical allodynia after 2-day guanethidine treatment starting 2 days after reperfusion (** $P < .01$, as compared with CPIP before drug; $n=6$ for each group). **B**, Effect of sympathetic block with guanethidine on mechanical allodynia in CPIP rats at 8 days after reperfusion. CPIP rats did not display a significant reduction in mechanical allodynia after 2-day guanethidine treatment starting 7 days after reperfusion ($n=6$ for each group). **C**, Effect of sympathetic block with guanethidine on mechanical allodynia in CPIP rats at 3, 5, 9, and 14 days after reperfusion. CPIP rats display a significant reduction in mechanical allodynia at 3, 5 and 14 days, after 2-day guanethidine treatment starting 2 days after reperfusion (* $P < .05$, ** $P < .01$, as compared with CPIP before drug; $n=6$ for guanethidine group, $n=7$ for vehicle group). I-R, ischemia-reperfusion.

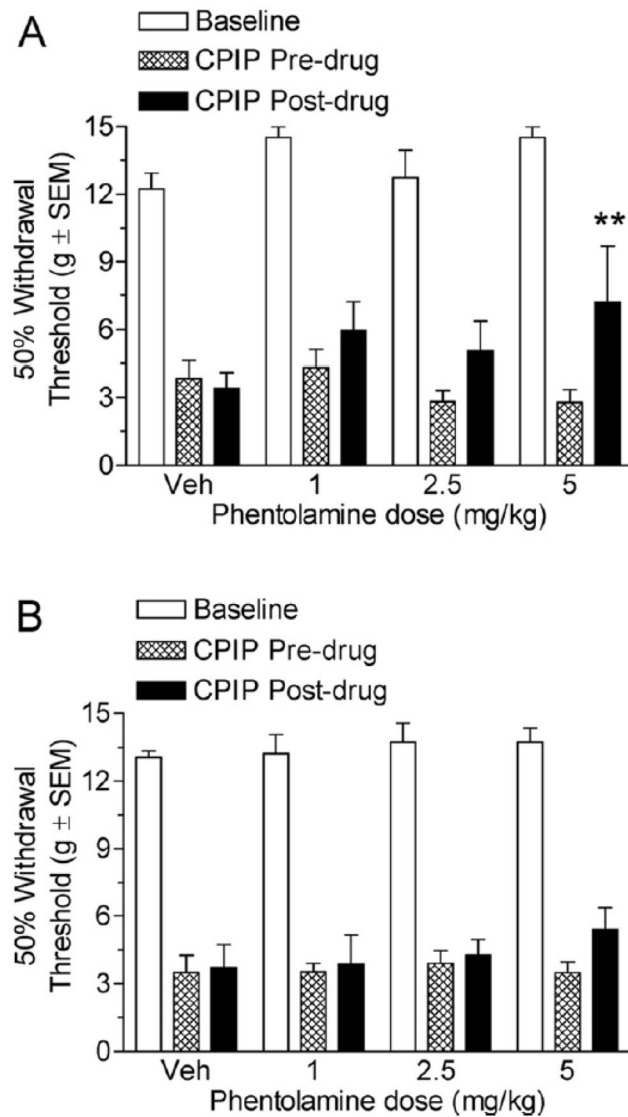


Figure 2.

A, Effect of systemic phentolamine in chronic postischemia pain (CPIP) rats at 2 days after reperfusion. CPIP rats display a significant reduction in mechanical allodynia with 5 mg/kg i.p. phentolamine administration but not with the 1 or 2.5 mg/kg doses (** $P < .01$ as compared with CPIP before drug; $n=6$ for each group). **B**, Effect of systemic phentolamine in CPIP rats at 7 days after reperfusion. CPIP rats did not display a significant reduction in mechanical allodynia with either 1, 2.5, or 5 mg/kg phentolamine doses at 7 days after reperfusion ($n=6$ for each group).

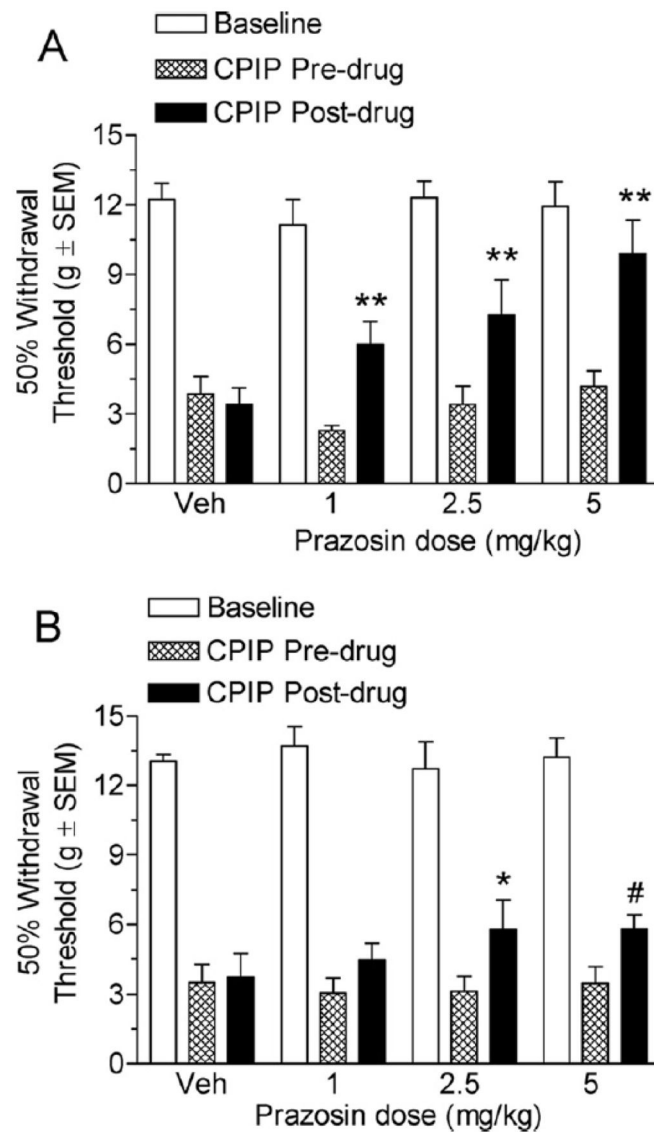


Figure 3.

A, Effect of systemic prazosin in chronic postschemia pain (CIP) rats at 2 days after reperfusion. CIP rats display a significant reduction in mechanical allodynia with 1, 2.5, and 5 mg/kg prazosin administration (** $P < .01$ as compared with CIP before drug; $n = 6$ for each group). **B**, Effect of systemic prazosin in CIP rats at 7 days after reperfusion. CIP rats display a significant reduction with the 2.5 and 5 mg/kg doses, but not the 1 mg/kg prazosin dose at 7 days after reperfusion (* $P < .05$ as compared with CIP before drug, # $P < .05$ as compared with CIP after vehicle; $n = 6$ for each group).

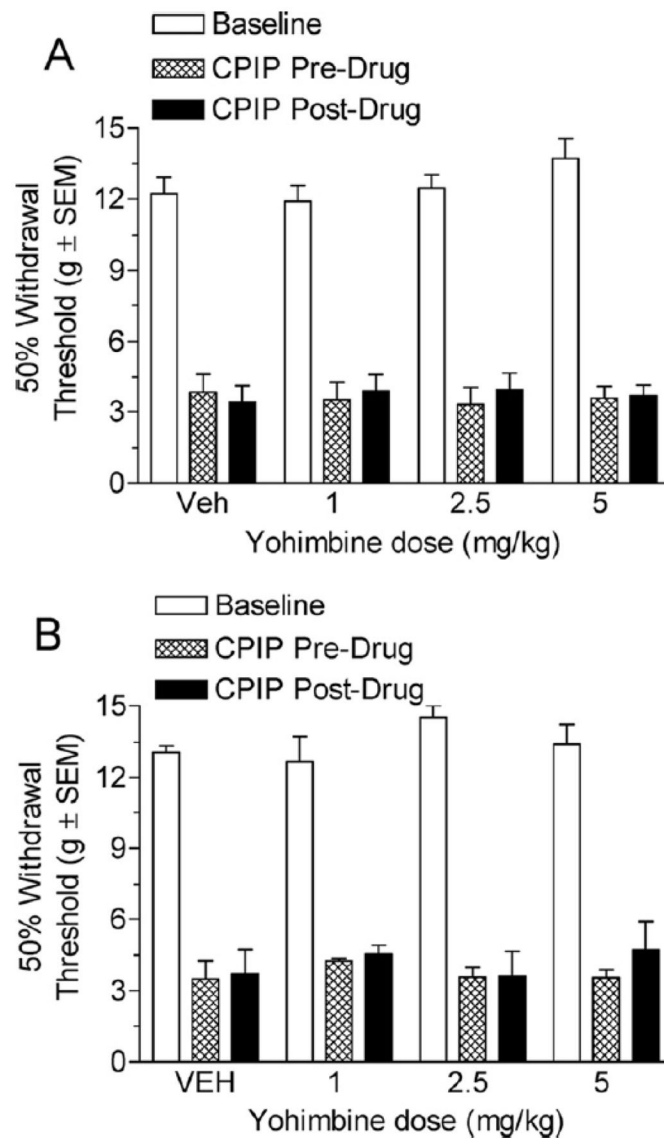


Figure 4.

A, Effect of systemic yohimbine in chronic postischemia pain (CPIP) rats at 2 days after reperfusion. CPIP rats did not display a significant reduction in mechanical allodynia with either the 1, 2.5, or 5 mg/kg yohimbine dose ($n=6$ for each group). **B**, Effect of systemic yohimbine in CPIP rats at 7 days after reperfusion. CPIP rats did not display a significant reduction in mechanical allodynia with either 1, 2.5, or 5 mg/kg yohimbine dose at 7 days after reperfusion ($n=6$ for each group).

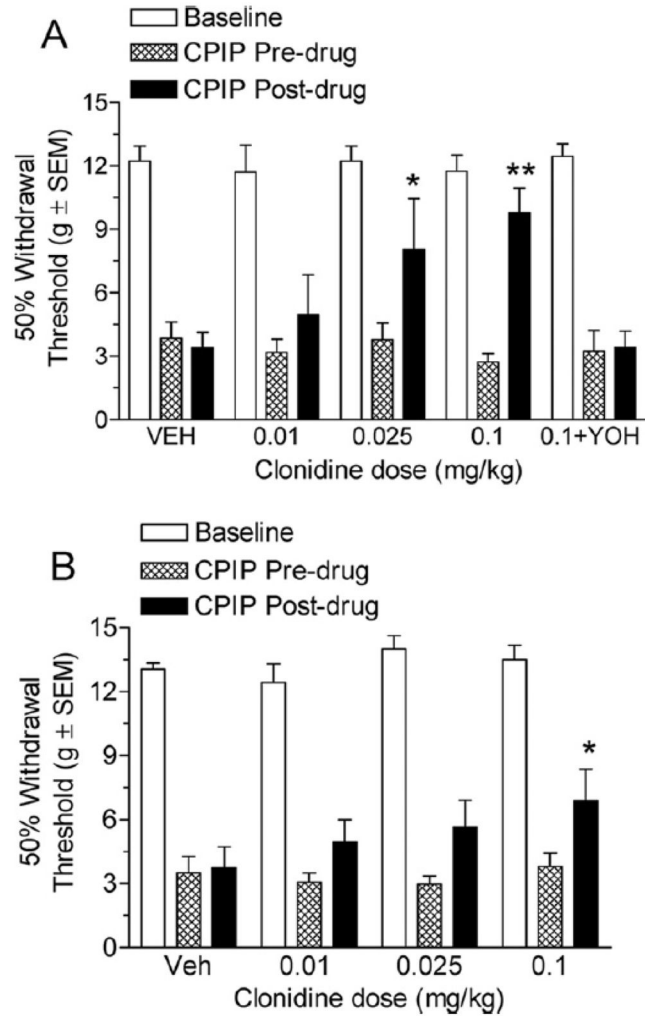


Figure 5. **A**, Effect of systemic clonidine in chronic postischemia pain (CPIP) rats at 2 days after reperfusion. CIP rats display significant reductions in mechanical allodynia with the 0.025 and 0.1 mg/kg clonidine doses but not with the 0.01 mg/kg dose. The effect of the highest dose of clonidine was reversed by yohimbine ($*P < .05$ and $**P < .01$, as compared with CIP before drug; $n = 6$ for each group). **B**, Effect of systemic clonidine in CIP rats at 7 days after reperfusion. CIP rats display a significant reduction in mechanical allodynia with a 0.1 mg/kg clonidine dose but not with the 0.01 or 0.025 mg/kg dose at 7 days after reperfusion. ($*P < .05$ as compared with CIP before drug; $n = 6$ for each group).

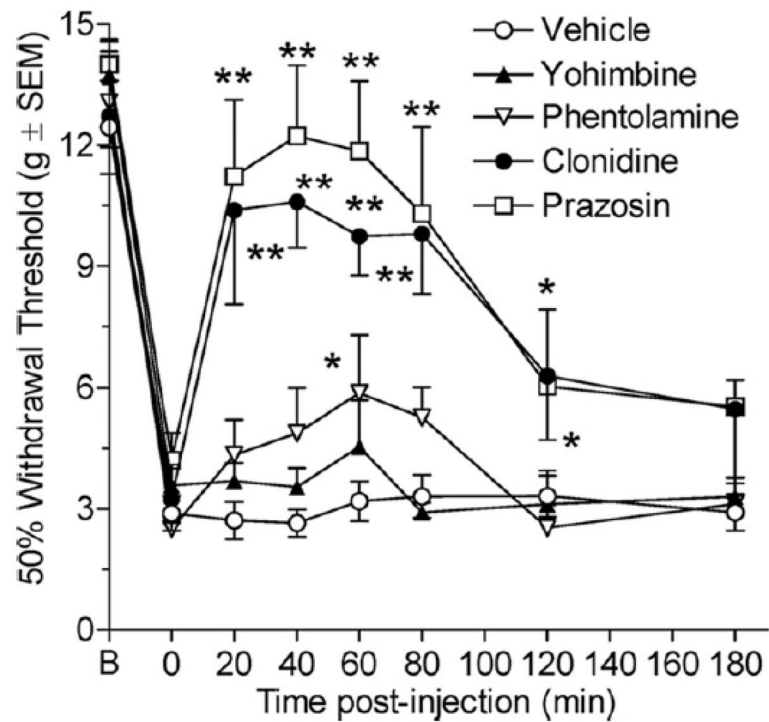


Figure 6.

Three-hour time course of anti-allodynic effects produced by systemic phentolamine, prazosin, yohimbine, and clonidine in 2-day chronic postischemia pain (CPIP) rats. Phentolamine (5 mg/kg) significantly reduced mechanical allodynia at 60 minutes after injection. Prazosin (5 mg/kg) significantly reduced mechanical allodynia at 20, 40, 60, 80, and 120 minutes after injection. Yohimbine (5 mg/kg) did not significantly reduce mechanical allodynia at any time measured. Clonidine (0.1 mg/kg) significantly reduced mechanical allodynia at 20, 40, 60, 80, and 120 minutes after injection (* $P < .05$ and ** $P < .01$, as compared with corresponding vehicle time point; $n = 6$ for each group).

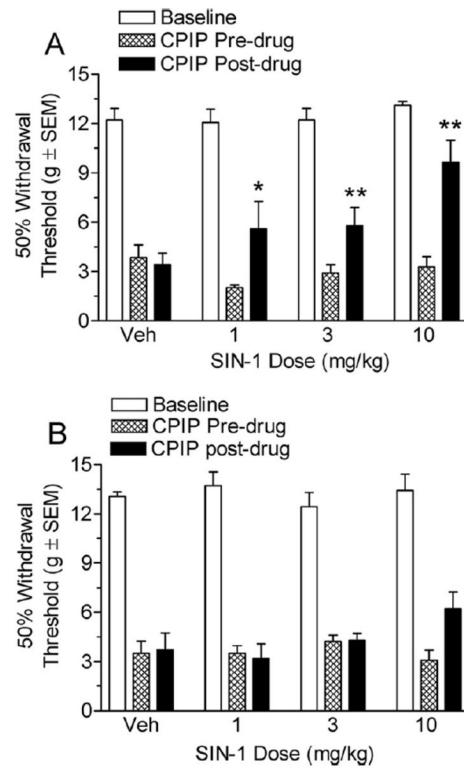


Figure 7.

A, Effect of systemic SIN-1 in chronic post-ischemia pain (CPIP) rats at 2 days after reperfusion. CPIP rats display significant reductions in mechanical allodynia with either 1, 3, or 10 mg/kg doses ($*P < .05$ and $**P < .01$, as compared with CPIP before drug; $n = 7$ for each group). **B**, Effect of systemic SIN-1 in CPIP rats at 7 days after reperfusion. CPIP rats did not display significant reductions in mechanical allodynia with either 1, 3, or 10 mg/kg of SIN-1 at 7 days after reperfusion ($n = 6$ for each group).