Topical prazosin attenuates sensitivity to tactile stimuli in patients with complex regional pain syndrome

Running head: Prazosin cream for complex regional pain syndrome

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What's already known about this topic?

- Expression of α_1-adrenoceptors is increased in the affected skin of patients with complex regional pain syndrome
- These receptors are blocked by the α_1-adrenoceptor antagonist prazosin

What does this study add?

- Topically-applied prazosin inhibits pinprick hyperalgesia in the CRPS-affected limb, and inhibits dynamic allodynia in patients with an adrenergic component of pain.

Abstract

Background: The sympathetic nervous system may play an important role in certain forms of chronic pain. The main aim of this study was to determine whether functional blockade of α_1-adrenoceptors would alter sensitivity to cutaneous stimulation in patients with complex regional pain syndrome (CRPS).

Methods and Results: In an initial study, high-performance liquid chromatography-mass spectrometry of intradermal interstitial fluid collected from the forearms of three healthy individuals established that the α_1-adrenoceptor antagonist prazosin penetrated the skin barrier when mixed in Lipoderm® cream base. Next, we found that application of this cream to the forearm of ten healthy participants attenuated axon reflex vasodilatation to the iontophoresis of phenylephrine, demonstrating functional blockade of α_1-adrenoceptors. Subsequently, effects of the cream on sensitivity to mechanical and thermal stimulation were investigated in 14 healthy participants and 19 patients with CRPS (eight with an apparent adrenergic component of pain). Both in patients and controls, topical application of the prazosin cream increased sensitivity to skin cooling but reduced sensations evoked by gentle brushing. In addition, hyperalgesia to sharp stimulation was lower at the prazosin- than vehicle-treated site in the CRPS-affected limb, and allodynia to brushing was lower at the prazosin- than vehicle-treated site in patients with an adrenergic component of pain.

Conclusions: Prazosin cream inhibited adrenergic axon reflex vasodilatation in healthy volunteers, and also inhibited dynamic allodynia and punctate hyperalgesia in the CRPS-affected limb of some patients. Further studies are required to assess the potential benefits of topically-applied prazosin for CRPS.

Introduction

Exposure to α_1-adrenoceptor (α_1-AR) agonists increases the firing rate of nociceptive neurons in animal models of neuropathic pain (Sato & Perl, 1991; Tracey *et al.*, 1995; Xie *et al.*, 1995; Petersen *et al.*, 1996; Maruo *et al.*, 2006; Meisner *et al.*, 2007; Xanthos *et al.*, 2008), and may intensify pain in patients with certain chronic pain syndromes (Davis *et al.*, 1991; Chabal *et al.*, 1992; Torebjork *et al.*, 1995; Choi & Rowbotham, 1997; Ali *et al.*, 2000; Mailis-Gagnon & Bennett, 2004; Lin *et al.*, 2006). Nevertheless, drugs that block these effects are not used widely in clinical practice, primarily due to concerns about systemic side-effects.

One way to circumvent systemic side-effects is to administer agents topically in low doses. Thus, the first aim of this study was to develop and test a topical preparation of prazosin (an α_1-AR antagonist) that was able to functionally block cutaneous α_1-AR. These receptors are activated by transcutaneous iontophoresis of the α_1-AR agonist phenylephrine, resulting in vasodilatation mediated by an axon reflex in the region surrounding the site treated with phenylephrine (Drummond, 2011). Therefore, to examine the functional effects of topical prazosin, we assessed the strength of axon reflex vasodilatation to iontophoresis of phenylephrine in the human forearm after treating the experimental site with prazosin cream. We hypothesized that pre-treatment with topical prazosin would inhibit the axon reflex to phenylephrine.

α_1-AR expression is increased in animal models of neuropathic pain (Drummond *et al.*, 2014a; Drummond *et al.*, 2014b) and in skin affected by complex region pain syndrome (CRPS) (Drummond *et al.*, 1996), specifically on cutaneous nerve fibres and keratinocytes (Drummond *et al.*, 2014b; Finch *et al.*, 2014). In a subset of patients with CRPS, intradermal injection of α_1-AR agonists such as noradrenaline and phenylephrine exacerbates pain around

the site of injection, and can rekindle pain that had disappeared after sympathetic blockade or sympathectomy (Davis *et al.*, 1991; Torebjork *et al.*, 1995; Ali *et al.*, 2000). Conversely, topical application of the α_2-AR agonist clonidine suppresses local hyperalgesia and pain (Davis *et al.*, 1991), possibly by acting on pre-synaptic α_2-AR autoreceptors to impede the neural release of noradrenaline and thereby decrease α_1-AR activation. Therefore, our second aim was to determine whether topical prazosin would inhibit hyperalgesia in CRPS patients. Specifically, we hypothesized that this treatment would reduce hyperalgesia in patients with an adrenergic component of pain.

Methods

Preliminary studies (AppendixS1)

An *in vitro* model of cutaneous absorption was used to determine whether aqueous, cetomacrogol or Lipoderm® bases allowed prazosin absorption through a 1 mm thick pig skin membrane. In addition, microdialysis was used to determine whether prazosin was absorbed topically into the forearm of three human volunteers. As absorption of prazosin was greatest from the Lipoderm® base cream, this formulation was used in all further studies.

Phenylephrine iontophoresis in healthy volunteers

Ten healthy volunteers (five women) provided informed consent for the procedures, which were approved by the Murdoch University Human Research Ethics Committee.

Prazosin hydrochloride (1% concentration) in Lipoderm® cream, and Lipoderm® cream without prazosin, were massaged into the skin with a cotton wool tip at sites several cm apart. Additional reservoirs of cream were contained inside 1 cm diameter flexible plastic washers over these sites, which were secured in place with Hypafix tape (Smith & Nephew Healthcare, London UK) for 24 hours. The forearms were then cleaned with soap and water,

and drugs were administered by iontophoresis using the method previously described (Drummond, 2011). Briefly, a Perspex iontophoresis capsule was placed directly over the area where the cream had been applied, and either phenylephrine hydrochloride (Sigma-Aldrich; 10 mM dissolved in de-ionized water) or saline was inserted into the capsule. Changes in blood flow were measured 8 mm from the outer rim of the iontophoresis chamber (in the region of neurogenic vasodilatation) using a Moor Instruments MBF3D laser Doppler flowmeter (Axminster, England). Baseline recordings of blood flow were first measured for two minutes. A 350 μA current was then passed through the phenylephrine or saline solution for three minutes to force positively-charged ions into the skin, and changes in blood flow were monitored for a further five minutes. Each participant underwent the following procedures: (1) iontophoresis of saline over skin with no cream applied; (2) iontophoresis of phenylephrine over skin with no cream applied; (3) iontophoresis of phenylephrine over skin treated with vehicle cream for one hour; (4) iontophoresis of phenylephrine over skin treated with 1% prazosin cream for one hour; (5) iontophoresis of phenylephrine over skin treated with vehicle cream for 24 hours; and (6) iontophoresis of phenylephrine over skin treated with 1% prazosin cream for 24 hours. The order of these procedures was randomised.

Prazosin treatment in CRPS patients and controls

The sample consisted of 16 women and three men aged 45.1 ± 2.3 years (mean ± S.E.) who met clinical and research criteria for CRPS in an upper or lower limb (Harden *et al.*, 2010), and nine female and five male pain-free controls aged 41.1 ± 4.2 years. Each participant provided informed consent for the procedures, which were approved by the Murdoch University Human Research Ethics Committee.

Each patient was reviewed by a pain physician to determine whether clinical and research criteria for CRPS were met (Harden *et al.*, 2010). The syndrome began after a limb fracture

(six cases, two with signs of peripheral nerve injury) or soft-tissue injury without obvious major nerve trunk involvement (ten cases), or after compression or laceration of a peripheral nerve (three cases). In each case, pain and other symptoms spread beyond the injured area, sometimes to another limb. Two patients reported mild contralateral symptoms and pain, but other patients with prominent bilateral symptoms were excluded. Patients were questioned about present or past sensory and autonomic disturbances in the affected limb, movement deficits and trophic changes in the hair, nails and skin. During the examination allodynia to briskly tapping the affected limb with a finger, swelling, and motor disturbances such as decreased range of movement, weakness and tremor, were assessed. In addition, trophic signs and autonomic disturbances were noted.

Assessment of an adrenergic component of pain in CRPS patients. Injection of α_1-AR agonists into healthy skin generally evokes transient pain lasting seconds, but occasionally generates more persistent pain not only when injected into the affected limb of CRPS patients but also when injected in the contralateral unaffected limb (Mailis-Gagnon & Bennett, 2004). Thus, to exclude potentially nonspecific effects of injection in CRPS patients, responses to injection of phenylephrine in the painful limb were compared with: (1) responses to phenylephrine in the contralateral unaffected limb (to determine the usual time course of pain evoked by intradermal injection of phenylephrine in asymptomatic skin in CRPS patients); and (2) responses to the α_2-AR agonist clonidine (which inhibits the neural release of noradrenaline, thereby suppressing pain in patients with an adrenergic component of pain) (Davis *et al.*, 1991). Clonidine also controlled for the vascular effects of phenylephrine, as both agents are potent vasoconstrictors.

To achieve this, patients attended the laboratory on two occasions separated by at least 24 hours (except for one patient who declined to participate in this part of the study). On each occasion, either 50 µg phenylephrine in 0.1 mL normal saline or 10 µg clonidine in 0.1 mL

normal saline were injected intradermally double-blind into a symptomatic area in the CRPS-affected limb and into a mirror-image site in the contralateral limb. Patients rated pain intensity at and around the site of injection between 0 (no pain) and 10 (extremely painful) at 5-minute intervals from before the injection to 40 minutes afterwards.

Patients were considered to have an adrenergic component of pain if: (1) increases in pain after intradermal injection of phenylephrine in the affected limb extended beyond 5 minutes (the usual duration of pain evoked by phenylephrine injected in the unaffected limb); and (2) pain decreased after injection of clonidine in the affected limb (to control for nonspecific increases in pain induced by an intradermal injection).

Prazosin treatment. On another occasion weeks or months later, prazosin and vehicle creams were applied as described above to 2 cm diameter sites in a symptomatic area of the CRPS-affected limb and to the contralateral unaffected limb of all patients, and to the left or right forearm or calf of the pain-free controls. The participant was unaware of which sites were treated with the active drug. Sites in the same limb were separated by several cm. Participants were asked to note whether they experienced any side-effects (particularly dizziness or faintness on standing) during the period of drug application.

Psychophysical assessment after the prazosin treatment. One day later participants attended a laboratory maintained at 22 ± 1^{o}C, where the prazosin and vehicle creams were removed and the sites cleaned with soap and water. Sensitivity to heat, cold, sharpness, pressure and brushing was then assessed at each site. For each modality, stimulation was rotated from one site to the next until all the sites had been tested. During these assessments, neither the experimenter nor the participant knew whether CRPS was associated with an adrenergic component of pain. To assess thermal thresholds, a thermode operating on the Peltier principle with a 2 cm diameter circular stimulating area was applied at a starting temperature

of 32^{o}C. The participant was asked to signal warm and cool detection and pain thresholds while probe temperature increased or decreased 0.5^{o}C /s to a maximum of 50^{o}C or a minimum of 0^{o}C. To assess sensitivity to sharpness, a sharp tip with a calibrated spring mechanism exerting a force of 40 g (Neuropen, Owen Mumford, USA) was applied for 2 s. Participants rated sharpness between 0 (none) and 10 (extreme). To measure sensitivity to pressure-pain, an algometer (FDX, Wagner Instruments, Greenwich, CT, USA) with a modified 10 mm diameter hemispheric rubber tip was applied at 100 g/s until the participant reported pain. Each site was then brushed gently with a soft brush (3–4 strokes backward and forward) and the participant was asked to describe what they felt (e.g., sharp, scratching, tingling or uncomfortable sensations, or dull or numb sensations).

Statistical approach

Phenylephrine iontophoresis. Changes in blood flow were measured in one-minute blocks during and after the iontophoreses, and were expressed as the percent change from levels during the preceding baseline. Effects of phenylephrine versus saline on neurogenic vasodilatation were analysed in Drug (phenylephrine, saline) x Time (eight consecutive minutes after the onset of the iontophoresis) repeated measures analyses of variance. A similar approach was used to investigate effects of the one-hour and 24-hour prazosin treatments on neurogenic vasodilatation to phenylephrine.

Intradermal injections of phenylephrine and clonidine. Changes in pain after the intradermal injections of phenylephrine and clonidine in the unaffected limb over Time (ratings taken at 5-minute intervals for 40 minutes) were analysed in repeated measures analyses of variance. To investigate differences between phenylephrine-responsive and phenylephrine-nonresponsive patients, an additional factor of Group was included in analyses for the CRPS-affected limb.

Prazosin treatment. Effects of the prazosin treatment on sensitivity to thermal and mechanical stimulation were investigated in Limb (CRPS-affected, contralateral, control) x Drug (prazosin, vehicle) analyses of variance, followed by simple contrasts to clarify significant effects. In addition, sensitivity to thermal and mechanical stimulation was investigated in Group (with versus without an adrenergic component of pain) x Side (CRPS-affected, contralateral) x Drug (prazosin, vehicle) analyses of variance.

Where appropriate, the Huynh-Feldt correction was used to correct for violations of the sphericity assumption. In all analyses, the criterion of statistical significance was $p < 0.05$.

Results

Phenylephrine iontophoresis in healthy volunteers

Iontophoresis of phenylephrine in ten healthy volunteers resulted in vasodilatation 8 mm from the site of phenylephrine iontophoresis whereas blood flow did not change after the saline iontophoresis [main effect for Drug $F(1,8) = 17.4$, $p<0.01$] (Fig. 1A). This suggests that the increased blood flow resulted from an axon reflex (neurogenic vasodilatation) in response specifically to phenylephrine and not as a result of stimulation from the electric current or iontophoresis of salts into the skin. The vasodilator response to phenylephrine was significantly attenuated after 24 hours of exposure to the prazosin cream [main effect for Drug $F(1,9) = 5.42$, $p<0.05$]; this was particularly notable in the latter stages of the response [Drug x Time interaction $F(2.08,18.69) = 3.74$, $p<0.05$] (Fig. 1B).Vasodilatation to phenylephrine was not affected by the one-hour application of prazosin (Fig. S1), suggesting that additional time was necessary for prazosin to enter the skin and have a functional effect.

Assessment of an adrenergic component of pain in CRPS patients

Intradermal injection of phenylephrine into the unaffected limb provoked brief but moderately intense pain at the site of injection that was significantly greater than baseline at 5 minutes but which had dissipated by 10 minutes [main effect for Time, $F(2.4, 40.9) = 20.5$, $p<0.001$] (Fig. 2A). Hence, after intradermal injection of phenylephrine into the CRPS-affected limb, persistently heightened pain at and around the injection site, that was at least 1 point greater on a 0-10 scale than at baseline during the 10-40 minute follow-up period, was considered abnormal. Based on this criterion, nine patients were phenylephrine-responsive and another nine patients were phenylephrine-nonresponsive. Pain evoked by phenylephrine persisted for most of the monitoring period in the phenylephrine-responsive patients but subsided rapidly in the other patients [Group x Time interaction, $F(3.2, 51.7) = 4.35$, $p<0.01$] (Fig. 2B).

Pain increased briefly after intradermal injection of clonidine into the unaffected limb [main effect for Time, $F(1.9, 31.7) = 15.2$, $p<0.001$] (Fig. 2C), and into the affected limb of the group as a whole [main effect for Time, $F(5.5, 87.5) = 12.8$, $p<0.001$] (Fig. 2D). The Group x Time interaction did not achieve statistical significance; however, it is worth noting that pain decreased below baseline at various points during the 40-minute follow-up period in phenylephrine-responsive patients (Fig. 2D). In particular, sensitivity and pain around the injection site decreased below baseline after the intradermal injection of clonidine in the affected limb of eight of the nine phenylephrine-responsive patients, thus meeting both criteria for an adrenergic component of pain.

Effects of prazosin cream on cutaneous sensitivity in CRPS patients and controls

None of the patients or controls reported side-effects after the application of the creams. However, sites treated with prazosin sometimes looked slightly pink, in line with local increases in blood flow associated with blockade of vascular α_1-AR.

Both with and without prazosin treatment, sensitivity to pressure-pain and sharpness was greater in the CRPS-affected limb of the group as a whole than in the contralateral limb or in controls [main effect for Limb: for pressure-pain, $F(2, 49) = 10.2$, $p<0.001$; for sharpness, $F(2, 49) = 11.0$, $p<0.001$], whereas sensitivity to skin cooling was diminished [main effect for Limb, $F(2, 49) = 3.97$, $p<0.05$] (Fig. 3). Both in patients and controls, sites treated with prazosin were more sensitive to skin cooling than vehicle-treated sites [main effect for Drug, $F(1,49) = 6.19$, $p<0.05$] (Fig. 3A). In addition, effects of the prazosin treatment on sharpness differed across limbs [Drug x Limb interaction, $F(2, 49) = 3.76$, $p<0.05$] (Fig. 3F). Further investigation of this interaction indicated that the prazosin pre-treatment reduced sensitivity to sharpness in the CRPS-affected limb ($p<0.05$) but had no effect on sharpness in the unaffected contralateral limb of patients or the pain-free limb of controls.

Brushing felt similar at both sites in nine controls (64%). However, the site pre-treated with prazosin was less sensitive to brushing than the site pre-treated with the vehicle cream in the other five controls (36%). Similarly, in the unaffected limb of CRPS patients, brushing felt similar at both sites in 15 cases. However, the site pre-treated with prazosin was less sensitive to brushing than the site pre-treated with vehicle cream in another 4 cases (21%). In no instance was the site pre-treated with prazosin *more* sensitive to brushing than the site pre-treated with the vehicle cream.

Brushing the affected limb evoked allodynia in seven patients with an adrenergic component of pain (i.e., 87.5% of this group), and in another seven patients without an adrenergic component of pain (70% of this group) (Table 1). The prazosin pre-treatment reduced allodynia to brushing in all seven patients with an adrenergic component of pain but had a similar effect in only two of seven patients without an adrenergic component of pain (i.e., 100% versus 28.6%, Fisher's Exact test $p<0.05$) (Table 1). The prazosin pre-treated site was

less sensitive to brushing than the vehicle-treated site in two of four patients without allodynia.

Influences of the prazosin pre-treatment on other forms of thermal and mechanical stimulation were similar in both groups.

Discussion

Topical application of the prazosin cream resulted in cutaneous absorption of prazosin (AppendixS1). This attenuated axon reflex vasodilatation to the iontophoresis of phenylephrine and altered skin sensitivity. These results support previous studies which suggested the presence of functional α_1-AR on cutaneous nerve fibres (Drummond, 1998; Lipnicki & Drummond, 2001; Drummond, 2009). Topical prazosin attenuated the phenylephrine-induced axon reflex at 24 hours but not at one hour after treatment. These *in vivo* results align with the *in vitro* results as notable prazosin absorption through a 1 mm thick pig skin membrane was not observed until 8 hours after treatment (AppendixS1).

Sensitivity to dynamic and punctate stimuli increases during whole body cooling in patients with sympathetically maintained pain (i.e., pain that decreases after sympathetic blockade or sympathectomy) (Baron *et al.*, 2002). Furthermore, direct intradermal injection of α_1-AR agonists increases pain and hyperalgesia in such patients (Davis *et al.*, 1991; Torebjork *et al.*, 1995; Ali *et al.*, 2000; Mailis-Gagnon & Bennett, 2004). Therefore, it follows that treatment with α_1-AR antagonists might be a therapeutic option for people affected by an adrenergic component of pain. The effectiveness of α_1-AR antagonists in animal models of pain is mixed (Tracey *et al.*, 1995; Lee *et al.*, 1999; Ringkamp *et al.*, 1999; Hord *et al.*, 2001; Kim *et al.*, 2005; Xanthos & Coderre, 2008; Hughes *et al.*, 2013), possibly because of variation across different animal species and whether the model induces an adrenergic component of pain. In several uncontrolled series of CRPS patients, pain decreased after oral administration of

nonspecific α-adrenergic antagonists (Ghostine *et al.*, 1984; Muizelaar *et al.*, 1997; Inchiosa & Kizelshteyn, 2008); furthermore, α_1-AR antagonists have been reported to be effective in individual patients (Abram & Lightfoot, 1981; Stevens *et al.*, 1993). However, α_1-AR antagonists are not prescribed routinely for treating CRPS. In our study, topical prazosin inhibited dynamic allodynia at the site of application without side effects in patients with an apparent adrenergic component of pain, possibly by blocking adrenergic actions on up-regulated α_1-AR in low-threshold, myelinated mechanoreceptors (Roberts, 1986; Dawson *et al.*, 2011; Drummond *et al.*, 2014b).

Both in patients and controls, the prazosin cream also inhibited innocuous sensations evoked by gently brushing the skin, possibly via the same mechanism. Sympathetic activation of slowly-adapting type 1 receptors in cats (Roberts *et al.*, 1985), and of Pacinian corpuscles in humans (Hallin & Wiesenfeld-Hallin, 1983), could indicate direct sympathetic modulation of activity in low-threshold, myelinated mechanoreceptors (Roberts, 1986). Similarly, sympathetic activation of A-delta nociceptors (Roberts & Elardo, 1985) might explain why prazosin inhibited hyperalgesia to stimulation with a sharp pin in the affected limb of patients with CRPS. It could be relevant that high-threshold A-mechano-heat receptors in the saphenous nerve of cats were activated by sympathetic stimulation only after they had been sensitized by noxious heating of their receptive fields (Roberts & Elardo, 1985), as this might account for the lack of effect of prazosin on sharp stimulation in the non-sensitized limbs of participants in our study. Individual variation in the intensity of sensitization in the CRPS-affected limb (mediated, for example, by growth factors or pro-inflammatory cytokines) (Birklein *et al.*, 2014) might also explain why inhibitory effects of prazosin on punctate hyperalgesia were detected not only in patients with an adrenergic component of pain but also in patients without this adrenergic component.

It could also be hypothesized that increases in blood flow due to release of vasoconstrictor tone after the prazosin treatment attenuated tactile sensitivity to gently brushing the skin. However, this seems unlikely as vasodilatation increases firing rates in slowly and rapidly adapting low-threshold cutaneous mechanoreceptors (Elam & Macefield, 2004). Nevertheless, increases in blood flow may account for heightened sensitivity to skin cooling in skin pre-treated with prazosin, as the warmth evoked by increased blood flow might have heightened the contrast to skin cooling.

CRPS is a rare condition that may encompass several interacting mechanisms which evolve over time. Thus, variation in uncontrolled factors (e.g., the type of injury, duration of pain, the extent of local inflammation or autonomic involvement, disuse of the affected limb, central sensitization, cortical plasticity, or psychological responses to pain) might have contributed to variation in the response to topically-applied prazosin in our small series of patients. This will need to be further explored in a larger sample to ensure adequate statistical power. In addition, variation in penetration through the skin barrier, or in the rate of dilution or metabolism of prazosin, might explain why the sensory effects of prazosin generally were small.

Several other limitations apply to our findings. For example, as the prazosin and vehicle creams were applied to sites several cm apart in the same limb, some effects of prazosin might have been masked by diffusion of prazosin from the treated to the control site. In addition, the study was not fully blinded as the prazosin site sometimes looked pink and the experimenter was aware of treatment allocation. Nevertheless, experimenter effects must have been small because most sensory modalities were unaffected by the prazosin treatment.

The prazosin treatment did not completely prevent the phenylephrine-induced axon reflex at 24 hours. It would be interesting in future studies to investigate the dose-response

relationship between the intradermal prazosin concentration and attenuation of the axon reflex, as it is possible that a cream with an increased concentration of prazosin would further attenuate, or even abolish, the development of the phenylephrine-induced axon reflex. Similarly, a higher concentration of prazosin cream (or a longer period or wider surface area of application) might more strongly inhibit sensations evoked by noxious stimulation of the skin.

Despite these limitations, specific inhibitory effects of the prazosin cream on adrenergic axon reflex vasodilatation, and on dynamic and punctate allodynia in the CRPS-affected limb, were identified in this small proof-of-concept study. Unfortunately, there are few other options for managing allodynia in this difficult group of patients. Ketamine cream can attenuate mechanical allodynia (Finch *et al.*, 2009), but its use is sometimes limited by systemic side effects. Therefore, larger double-blind placebo-controlled trials of topical anti-adrenergic agents, such as prazosin, for inhibiting allodynia in CRPS seem warranted.

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Figure legends

Figure 1. Percent change ± S.E. in skin blood flow during and after three minutes of iontophoresis. (A) Increases were greater after the iontophoresis of phenylephrine than saline. (B) Topical application of prazosin for 24 hours inhibited neurogenic vasodilatation to phenylephrine. Differences between drug conditions statistically significant: * $p<0.05$; ** $p<0.01$.

Figure 2. Pain ratings (± S.E.) after intradermal injection of α-adrenergic agonists into the unaffected and affected limbs of 18 patients with CRPS. (A) Pain increased significantly above baseline for 5 minutes after 50 µg phenylephrine was injected into the unaffected limb (* $p<0.05$ compared with baseline). (B) Intradermal injection of phenylephrine into the CRPS-affected limb provoked a prolonged increase in pain in nine phenylephrine-responsive patients (* $p<0.05$ compared with baseline). However, pain quickly returned to baseline in the rest of the group (N = 9). (C) Pain increased briefly after 10 µg clonidine was injected into the unaffected limb (* $p<0.05$ compared with baseline). (D) In phenylephrine-responsive patients, intradermal injection of clonidine into the CRPS-affected limb provoked a brief increase in pain followed by a prolonged decrease in pain (* $p<0.05$ compared with baseline). However, pain quickly returned to baseline in the rest of the group.

Figure 3. Sensitivity to thermal and mechanical stimulation at sites treated with 1% prazosin cream or vehicle (± S.E.) in19 patients with CRPS and 14 controls. (A) Both in patients and controls, sites treated with prazosin were more sensitive to cooling than vehicle-treated sites. In addition, the cool detection threshold was diminished in the CRPS-affected limb. (B) The cold-pain threshold did not differ between Limbs or Drug conditions. (C) The CRPS-affected limb was sensitive to pressure-pain. However, prazosin had no effect on the pressure-pain threshold. (D) and (E) Neither the warm detection threshold nor the heat-pain threshold differed between Limbs or Drug conditions. (F) Sharpness was greater in the CRPS-affected limb than in contralateral or control limbs. However, sharpness was tempered in the CRPS-affected limb by pre-treatment with prazosin. Difference between the prazosin and vehicle conditions statistically significant for the CRPS-affected limb: * $p<0.05$.

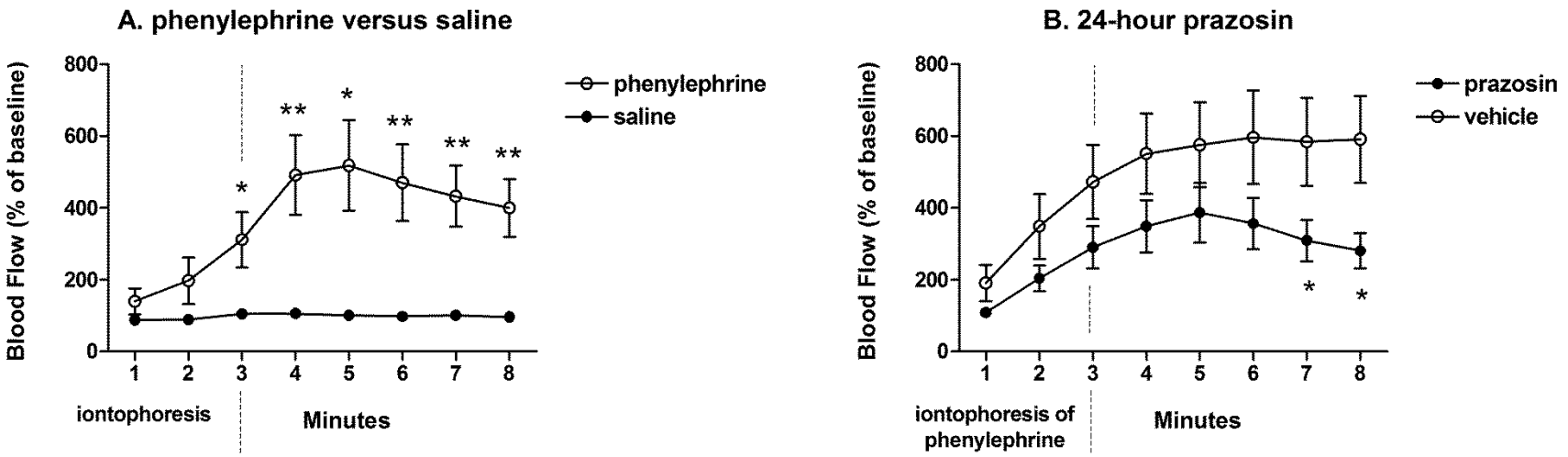
Figure S1. Percent change ± S.E. in skin blood flow during and after three minutes of phenylephrine iontophoresis at sites treated for one hour with prazosin or vehicle cream.

Table 1

Effect of 1% prazosin cream on sensitivity to brushing the affected limb in patients with and without an adrenergic component of pain

	Sensations induced by brushing	
	Vehicle-treated site	**Prazosin-treated site**
Patients with an adrenergic component of pain		
1.	Irritation, discomfort	No sensation
2.	Sharp	Dull sensation
3.	Tingling	Decreased sensation, soft
4.	Slightly sharp	Decreased sensation, barely felt
5.	Tingling	Normal brush
6.	Burning sensation that builds	Dull, no after-sensation
7.	Localized strong pain	Diffuse pain
8.	Normal brush	Normal brush
Patients without an adrenergic component of pain		
1.	Very sharp	Sharp
2.	Dull pain	Normal brush
3.	Normal brush	Numb
4.	Normal brush	Normal brush
5.[a]	Decreased sensitivity	Greatly decreased sensitivity
6.	Tingling	Intense tingling
7.	Pins-and-needles	Pins-and-needles
8.	Firm blunt sensation	Sensitive, ticklish
9.	Painful	Very painful, spreads
10.	Ticklish, heavy	Rough, painful

[a]In this patient pain increased in the affected limb both after the intradermal injection of phenylephrine and the intradermal injection of clonidine, and thus was considered not to have an adrenergic component of pain.



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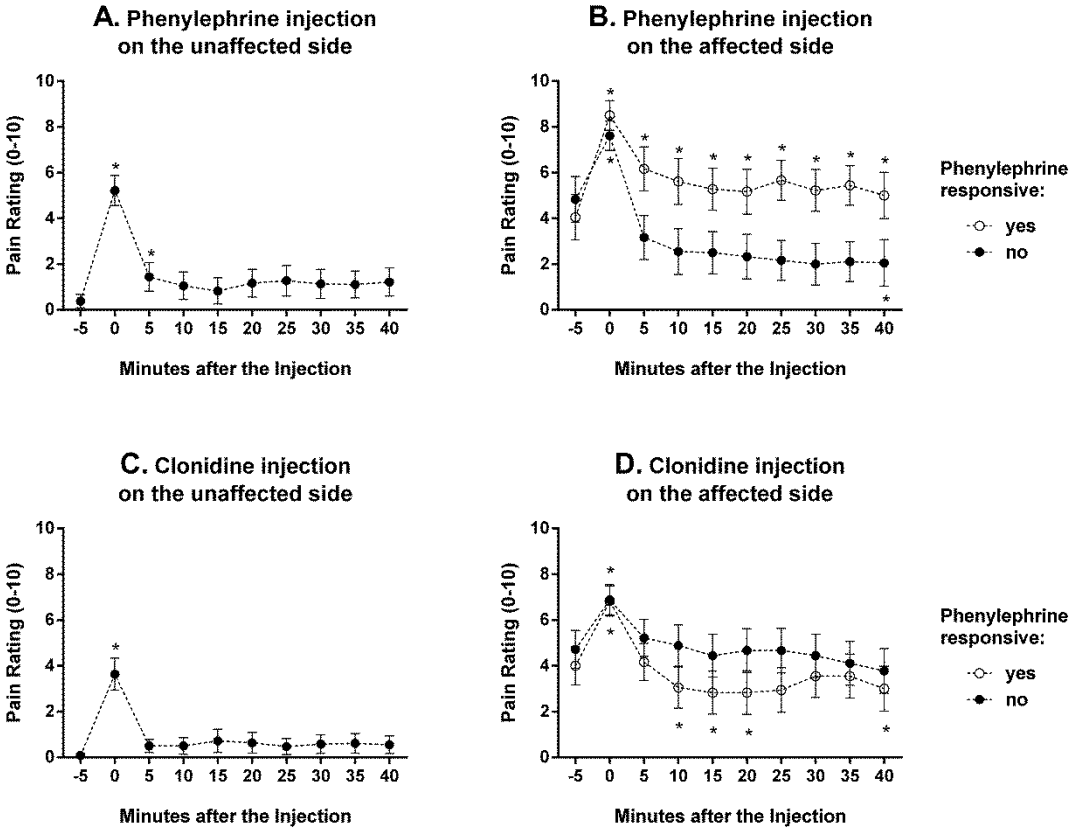

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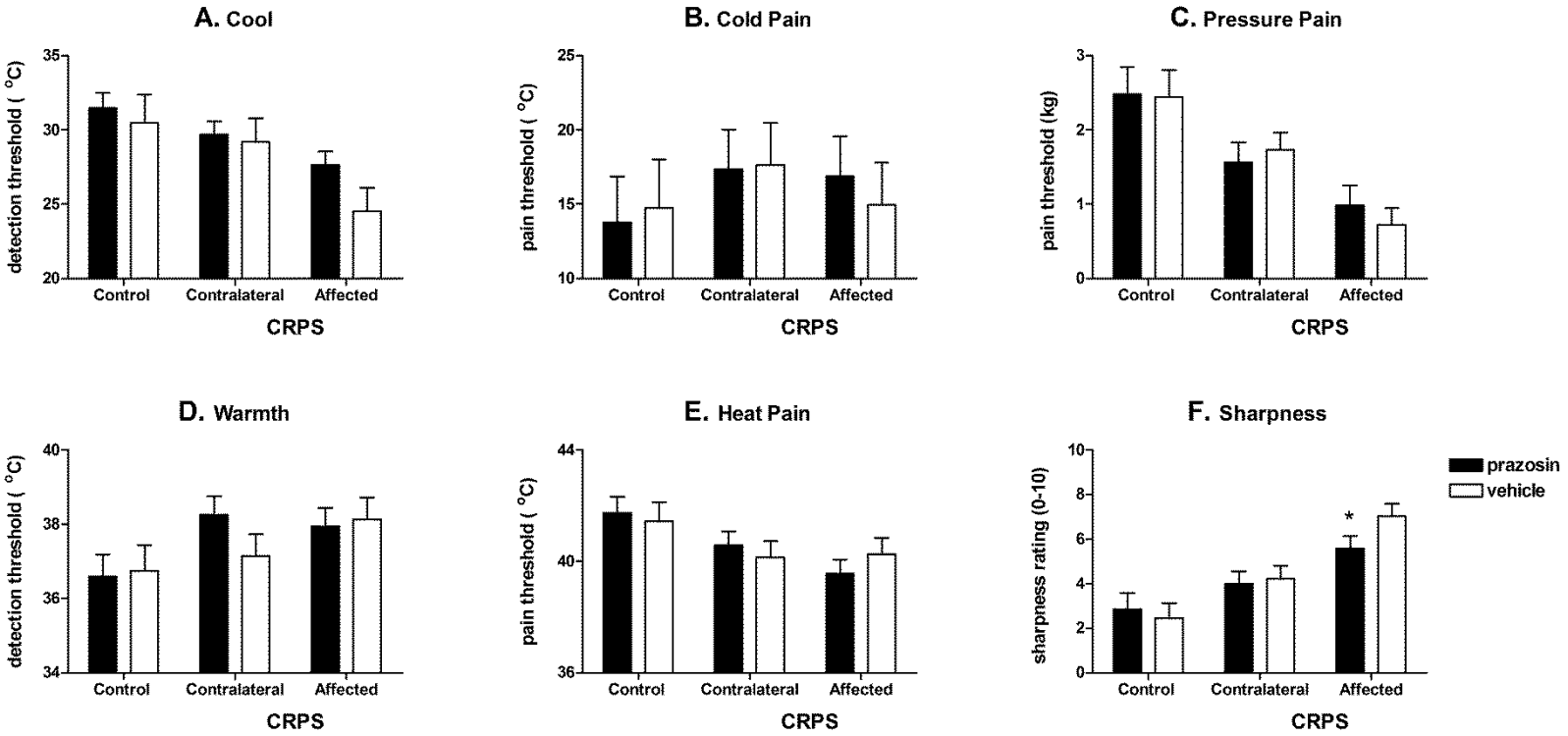

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Appendix S1

Topical prazosin absorption

Initial studies were performed to test whether prazosin was more effectively absorbed into the skin when compounded in an aqueous, cetomacrogol or Lipoderm® base (PCCA, Houston, Texas). Prazosin hydrochloride (Sigma-Aldrich, St. Louis, USA) was dissolved in each of these bases at a concentration of 0.5%. Sections of pig skin 1 mm thick were used as a permeable membrane in a modified Ussing chamber. Prazosin was applied to the epidermal side of the pig skin and prazosin absorption was examined in phosphate-buffered saline on the dermal side at 1, 2, 4, 8, 24 and 48 hours after application using liquid chromatography–mass spectrometry (LC-MS).

LC-MS-grade water, methanol and acetonitrile were obtained from Honeywell Burdick and Jackson (Muskegon, USA). Formic acid and diphenhydramine (internal standard) were obtained from Sigma-Aldrich (St. Louis, USA). Due to low solubility in water, prazosin standards were first dissolved in methanol and subsequently diluted into 95% water and 5% acetonitrile with 1% formic acid, representing the initial high-performance liquid chromatography (HPLC) mobile phase composition. Standards were analysed over the concentration range 0.1 to 1000 ng/mL. Diphenhydramine was spiked into all samples at a concentration of 50 ng/mL as an internal standard.

Samples were analysed on a Varian 212-LC with PAL autosampler. The column used was a Restek Ultra Aqueous C_{18} (100 x 2.1 mm, 3µm). The flow rate was 0.2 ml/min and the mobile phase consisted of: A: H_2O + 1% formic acid; B: acetonitrile + 1% formic acid. The HPLC gradient was as follows (% A:B): 0 min – 95:5; 2 min – 95:5; 9 min – 0:100; 14 min – 0:100; 15 min – 95:5; 20 min – 95:5. The HPLC was coupled to a Varian 325-MS triple quadrupole mass spectrometer with a vortex electrospray ionisation source (Agilent

Technologies, USA). Drying gas was set at 300°C and 25 psi, nebulizing gas was set at 70 psi and vortex gas was set at 300°C and 30 psi. Capillary voltage was set to 30 V in positive ionisation mode and CID gas (argon) pressure was 2 mTorr. Automated MS/MS breakdown was performed on the prazosin standard to determine the appropriate transitions for analysis. Three transitions, in positive ion mode, were identified: *m/z* 384 → 136, 231 and 247. For diphenhydramine internal standard, the following transitions were used: *m/z* 256 → 152 and 167. LC-MS data was analysed using Varian Workstation v. 7.0 (Agilent Technologies, USA), normalized to diphenhydramine peak area. Linearity was confirmed by plotting a standard curve of peak area versus drug concentration over 0.1 to 500 ng/mL. Sensitivity was determined as a limit of detection with a signal-to-noise ratio not less than 3:1 and a limit of quantification with a ratio of not less than 10:1. Concentration values below the limit of quantification were set at zero.

Prazosin absorption from the aqueous cream base was first observed 1 hour after application, and 8-24 hours after application this had plateaued at 61-63 ng/mL. The cetomacrogol base resulted in notable prazosin absorption between 4 and 8 hours after application, which plateaued 24 hours after application at 62 ng/mL. The Lipoderm® base resulted in the greatest absorption of prazosin; notable prazosin absorption was first observed between 4 and 8 hours after application, and this increased steadily to 353 ng/mL 24 hours after application and 585 ng/mL at 48 hours. Therefore, the Lipoderm® base cream was used for all further experiments.

Analysis of cutaneous prazosin absorption in the human forearm

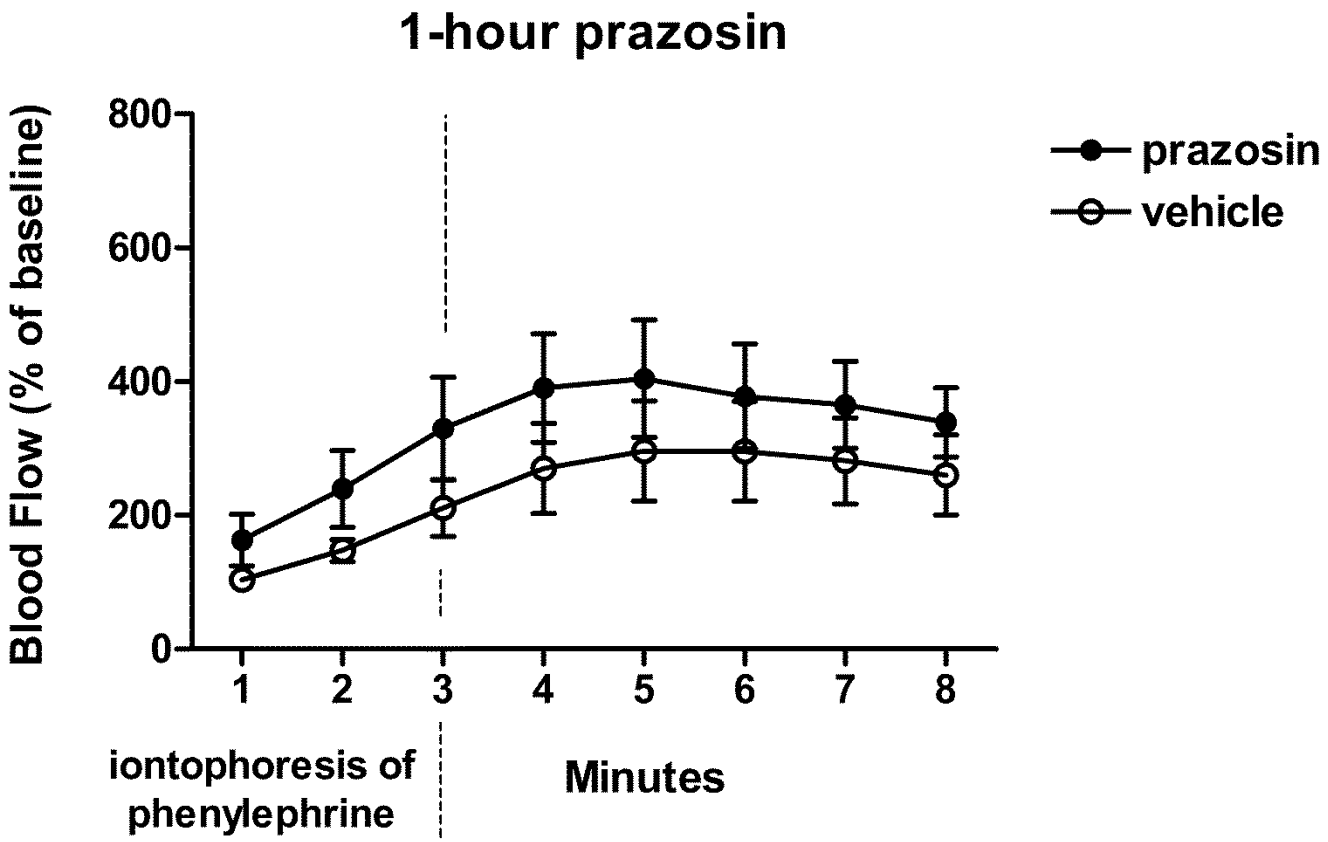
The cutaneous absorption of prazosin in the healthy human forearm was examined in three participants who provided their informed consent for the procedures, which were approved by the Murdoch University Human Research Ethics Committee.

Cream containing 1% prazosin hydrochloride, or cream without prazosin, was massaged into the skin at sites several cm apart with a cotton wool tip. A 1% concentration of prazosin was used as this concentration mixed readily into the Lipoderm® base and was more likely to produce functional effects than lower concentrations. Storage capsules (1 cm diameter) containing additional reservoirs of cream were secured over the sites for 24 hours. The creams were then removed and the forearm was cleaned with soap and water. In two of the three participants, prazosin cream was applied at this stage to the area treated with vehicle cream and immediately removed with soap and water, to control for prazosin contamination on the surface of the skin.

The intradermal interstitial prazosin concentration was examined using dermal microdialysis. The skin on the left forearm was cooled using an ice pack to produce analgesia. Four hollow microdialysis fibres (0.4 mm in diameter, cut off 3000 kDa; DermalDialysis, Erlangen, Germany) were then inserted intracutaneously at a depth of approximately 0.65 mm over a length of 15 mm through 25-gauge cannulae; two under the area treated with prazosin cream and two under the area treated with vehicle cream. Saline was perfused through the fibres at 4 μL/min using a microdialysis pump (Pump 22/2000, Harvard Apparatus). The dialysis eluate was collected for an hour and then stored at -80°C. Liquid chromatography–mass spectrometry was used to determine the prazosin concentration in the eluate using the method described above.

Prazosin was detected in the dialysis eluate of all three participants after 24 hours of topical application. In two of the three participants, the concentration of prazosin was twice as high in the area treated with prazosin cream than in the area treated with vehicle cream (94 ng/mL and 47 ng/mL compared with 40 ng/mL and 26 ng/mL, respectively). In both cases, prazosin cream had been applied briefly to the area treated with vehicle cream to exclude the possibility that higher values in the microdialysis eluate at the prazosin-treated site merely

reflected prazosin contamination on the surface of the skin. In the third participant, prazosin was 49 ng/mL at the prazosin site compared with 44 ng/mL at the site treated with vehicle cream. Together, these findings indicate that the prazosin concentration was greatest at the site of entry into the skin with minor diffusion away from this site, and that brief application of prazosin cream at the area treated with vehicle cream did not increase the prazosin concentration at this site.



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