Depletion of Noradrenaline Inhibits Electrically-Evoked Pain in the Skin of the Human Forearm

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Abstract

Guanethidine displaces noradrenaline from sympathetic varicosities, and blocks sympathetic noradrenergic neurotransmission by inhibiting the release of noradrenaline from depleted neural stores. The aim of this study was to determine whether depletion of noradrenaline with guanethidine would oppose thermal hyperalgesia and/or electrically-evoked pain in mildly-burnt skin. Guanethidine was transferred by iontophoresis into a small patch of skin on the forearm of 35 healthy human subjects. The heat pain threshold to a temperature gradient that increased at 0.5^{o}C/s was then measured at the guanethidine site, a nearby saline-control iontophoresis site, and in untreated skin. In addition, participants rated pain intensity to a 47^{o}C stimulus that was applied to each site for 7 s. Shortly after the iontophoreses, sensitivity to heat was greater at the guanethidine site than the two control sites, suggesting that ejection of noradrenaline from sympathetic varicosities increased sensitivity to heat. One day later, when neural stores of noradrenaline were depleted, sensitivity to heat did not differ between the guanethidine and control sites. The guanethidine pretreatment did not influence thermal hyperalgesia induced by a mild burn, but inhibited pain evoked by electrical stimulation of the skin (0.2 mA direct current for 4 minutes). These findings indicate that ongoing sympathetic neural discharge does not normally influence thermal hyperalgesia in inflamed skin, because depleting noradrenergic stores had no effect. However, electrically-evoked release of noradrenaline may increase nociceptive sensations. Further clarification of this human pain model could provide insights into the mechanism of adrenergic hyperalgesia in certain neuropathic pain syndromes.

Introduction

Sympathetic neural discharge does not normally excite primary nociceptive afferents. However, electrophysiological experiments in animals have demonstrated that primary afferent nociceptors develop sensitivity to adrenergic agents during inflammation and after nerve injury. For example, after nerve transection, stimulation of the sympathetic chain and local injection of α-adrenergic agonists excites sensory fibres within the resultant neuroma (Burchiel, 1984). Adrenergic agonists also excite dorsal root fibres excised two weeks after chronic loose constriction of the sciatic nerve (Zhang et al., 1997), and excite uninjured fibres that survive partial destruction of the sciatic nerve (Sato and Perl, 1991; Ali et al., 1999). Similarly, stimulation of the sympathetic chain and intra-arterial injection of adrenergic agonists provokes hyperalgesia and discharge of primary nociceptive afferents in inflamed tissue (Hu and Zhu, 1989; Sato et al., 1993; Baik et al., 2003). Conversely, blockade of α_1-adrenergic receptors blocks C-fibre discharge to mechanical stimulation of cutaneous fibres sensitized by intradermal injection of capsaicin (Ren et al., 2005).

Studies in humans also indicate that adrenergic agents influence hyperalgesia in inflamed skin. For example, blockade of α-adrenergic receptors inhibits ongoing and mechanically-evoked pain in skin sensitized by an intradermal injection of capsaicin (Kinnman et al., 1997). Conversely, thermal hyperalgesia is augmented by iontophoresis of noradrenaline into skin made sensitive to heat by the topical application of capsaicin (Drummond, 1995). This adrenergic hyperalgesia is blocked by α-adrenergic antagonists and persists after arterial occlusion (Drummond, 1996; Drummond, 1998a; Drummond, 1999).

Tyramine displaces noradrenaline from the synaptic vesicles and cytoplasm of sympathetic nerve fibres (Smith, 1973), and augments sensitivity to heat in skin

already sensitized by the topical application of capsaicin (Drummond, 1998b). Like tyramine, guanethidine displaces noradrenaline from sympathetic varicosities (Chang et al., 1965), but guanethidine also blocks sympathetic noradrenergic neurotransmission by activating potassium channels in the neural membrane (Fabiani and Story, 1996). Displacing noradrenaline from sympathetic varicosities with guanethidine takes around three hours (McKain et al., 1983), and replenishing these neural stores requires several days (Lipnicki and Drummond, 2001).

In a previous study in our laboratory, repeated administration of guanethidine over a two-week period induced vascular signs of adrenergic supersensitivity (Lipnicki and Drummond, 2001), presumably due to an up-regulation of adrenergic receptors during prolonged sympathetic blockade. In addition, the guanethidine pretreatment augmented thermal hyperalgesia induced by the iontophoresis of noradrenaline in capsaicin-treated skin, suggesting that stimulation of the up-regulated adrenergic receptors intensified sensitivity to heat.

The aim of the present study was to extend these findings by investigating the short-term effects of guanethidine on thermal hyperalgesia. In particular, it was hypothesized that an initial efflux of noradrenaline into the skin, provoked by guanethidine, would increase sensitivity to heat. To test this hypothesis, sensitivity to heat was investigated shortly after guanethidine was transferred by iontophoresis into a small patch of skin on the forearm of healthy human subjects. One day later, when neural stores of noradrenaline were depleted, sensitivity to heat was investigated before and after heat-sensitization induced by a mild burn, to determine whether depletion of noradrenaline at the guanethidine-pretreated site would inhibit sensitivity to heat. In some cases the heat-sensitized skin was also stimulated electrically with a 0.2 mA direct current. It was hypothesized that electrically-evoked release of

noradrenaline from sympathetic nerve terminals would augment nociceptive sensations, and that adrenergic depletion with guanethidine would block this effect.

Method

Subjects

Participants were aged between 18 and 58 years and were free of chronic pain or any other major medical condition. Each subject provided their informed consent for the procedures, which were approved by the Murdoch University Human Research Ethics Committee.

Procedure

The procedures were administered in a temperature-controlled room maintained at 21 $\pm$ 1^{o}C. In the first session, the immediate effect of local guanethidine administration on sensitivity to heat was compared with sensitivity to heat at a saline-control site and an untreated site in the same forearm (N=35). The effect of depleting noradrenaline on sensitivity to heat before and after a mild burn was investigated in a second session one day later in 25 of these subjects. In the last 16 subjects tested, the effect of the guanethidine pretreatment on sensations induced by electrical stimulation was also investigated. In previous studies in our laboratory (e.g., Drummond, 1995; Drummond, 1996; Drummond, 1998a; Drummond, 1998b; Drummond, 1999; Lipnicki and Drummond, 2001), significant effects of noradrenaline and guanethidine on sensitivity to heat were identified in samples of 20 or fewer subjects. Thus, the sample size in the present study was considered to be large enough to provide an adequate test of the hypotheses.

Session 1: guanethidine administration. Guanethidine hydrochloride (Sigma Chemical Company, Sydney, Australia) was dissolved in de-ionized water to form a 10 mM solution. In the first session, guanethidine ions were transferred by

iontophoresis from the 10 mM solution into a small patch of skin on the participant's right or left forearm. To investigate any nonspecific effects of iontophoresis, a control procedure involving iontophoresis of 0.9% saline was also carried out at another site in the forearm. The site location and order of guanethidine and saline iontophoreses were randomized across subjects. The iontophoresis chambers had an internal diameter of 2 cm, and were fixed at least 10 cm apart on the forearm with adhesive washers. A constant current of 0.1 mA simultaneously passed through the guanethidine and saline solutions in the chambers for 30 minutes, to repel positively-charged ions from the solution into the underlying skin. To complete the electric circuits, a cathode was attached to the back of the hand. The dose of guanethidine delivered by this current blocks local vasoconstriction to tyramine for several days (Lipnicki and Drummond, 2001), consistent with neural depletion of noradrenaline (Chang et al., 1965; Fabiani and Story, 1996).

Session1: assessment of sensitivity to heat. Fifteen minutes after the iontophoreses, sensitivity to heat was measured at the guanethidine site and at two control sites (the saline site and untreated skin several cm away from the other sites). Heat was delivered from a custom-built servo-controlled thermode containing a Peltier element (contact diameter 2 cm). The thermode was held against the skin for 10-15 s to bring the skin temperature to 32^{o}C, then increased at 0.5^{o}C/s to a maximum of 49^{o}C or to the heat-pain threshold. Participants were informed that sensations at the site of stimulation would change from warmth to heat, and then to stinging or burning pain. They were instructed to press a key to prevent further heating at the onset of pain. The heat pain threshold was calculated from three temperature ramps at each site. The thermode was then heated to 47^{o}C and applied once at each site for 7 s. Participants rated pain intensity verbally between 0 and 10 where 0 corresponded to

"not painful", 5 to "moderately painful", and 10 to "extremely painful". Sensitivity to heat was investigated once more at each site using the same procedures after an interval of 15 minutes, to investigate the stability of responses.

Session 2: assessment of sensitivity to heat before and after a mild burn. When participants returned to the laboratory one day later, heat pain thresholds were measured as described above at the guanethidine site and the two control sites (saline and untreated skin). Participants also rated the intensity of pain induced by a 45^{o}C stimulus applied once at each site for 7 s (45^{o}C was used instead of 47^{o}C to avoid ceiling effects on pain ratings later in the session). Next, the thermode was heated to 48^{o}C, and pain intensity was monitored while the thermode was applied to one of the three sites (tested in random order) for two minutes to induce a mild burn. Thirty minutes later, the heat pain threshold and pain ratings to the 45^{o}C stimulus were obtained at that site. The same procedures were repeated at the other two sites.

Session 2: assessment of electrically-evoked sensations. For each site in turn, a 0.2 mA direct current was delivered from an iontophoresis chamber (2 cm diameter) filled with 0.9% saline. Pain induced by the electric current was rated between 0-10 at one-minute intervals for four minutes.

Data analysis

The immediate effect of the guanethidine iontophoresis on heat pain thresholds and heat pain ratings in Session 1 was investigated in 3 x 2 [Site (guanethidine, saline, untreated) x Time (15 and 30 minutes after the iontophoreses)] repeated measures analyses of variance. To avoid violations of the sphericity assumption, the multivariate solution was used for effects with more than two levels (Vasey and Thayer, 1987).

The delayed effect of the guanethidine iontophoresis on heat pain thresholds and heat pain ratings in Session 2 was investigated in 3 x 2 [Site (guanethidine, saline, untreated) x Time (before and after heating the skin to 48^{o}C)] repeated measures analyses of variance. Mean pain ratings during the 48^{o}C stimulus and pain ratings during each minute of electrical stimulation were compared across the three sites in repeated measures analyses of variance. In the latter analysis, the Greenhouse-Geisser epsilon was applied to the degrees of freedom to correct for violations of the sphericity assumption, because *N* was too small to use the multivariate approach (Vasey and Thayer, 1987).

The criterion of statistical significance was $p<0.05$. Significant main effects and interactions were investigated with *a priori* (i.e., "planned") contrasts between the guanethidine site (the experimental site) and each of the two control sites. Data are reported as the mean $\pm$ standard error.

Results

Session1: Immediate effect of the guanethidine iontophoresis

As shown in Figure 1A, the heat pain threshold was lower at the guanethidine site than at the saline site or in untreated skin [main effect for Site, $F(2,33) = 40.2$, $p<0.001$; mean difference between the guanethidine and untreated sites 2.5 $\pm$ 0.3^{o}C, $F(1,34)=77.6$, $p<0.001$; mean difference between the guanethidine and saline sites 2.3 $\pm$ 0.3^{o}C, $F(1,34)=76.7$, $p<0.001$]. The heat pain threshold increased during the 15 minute interval between measures, particularly at the guanethidine site [Site x Time interaction, $F(2,33)=16.7$, $p<0.001$]. However, recovery was not complete – the heat pain threshold remained lower at the guanethidine site than at the other two sites at the end of the session [mean difference between the guanethidine and untreated sites 2.0 $\pm$ 0.3^{o}C, $F(1,34)=58.4$, $p<0.001$; mean difference between the guanethidine and saline

sites 1.8 ± 0.3°C, $F(1,34)=46.8$, $p<0.001$]. The heat pain threshold did not differ significantly between the saline and untreated sites.

As shown in Figure 1B, pain ratings to the 47°C stimulus averaged 7.2 (corresponding to between moderately and extremely painful) at the guanethidine site compared with 5.4 at the saline site and 5.0 in untreated skin [main effect for Site, $F(2,33) = 37.5$, $p<0.001$; mean difference between the guanethidine and untreated sites 2.2 ± 0.3, $F(1,34)=68.2$, $p<0.001$; mean difference between the guanethidine and saline sites 1.8 ± 0.2, $F(1,34)=68.5$, $p<0.001$]. In addition, pain ratings were slightly greater at the saline site than in untreated skin [mean difference between the saline and untreated sites 0.4 ± 0.2, $F(1,34)=5.14$, $p<0.05$]. Ratings did not change significantly during the 15 minute interval between tests.

Session 2: Effect of the guanethidine iontophoresis one day later

Sensitivity to heat did not differ significantly among the three sites before the burn induction (Figure 2), indicating that thermal hyperalgesia at the guanethidine-pretreated site had subsided. Pain ratings averaged 7.6 ± 0.3 during the 48°C burn, and were similar at each site. An increased sensitivity to heat was detected at all three sites 30 minutes after the 48°C stimulus [mean decrease in the heat pain threshold 1.5 ± 0.4°C, $F(1,24)=16.7$, $p<0.001$; mean increase in heat pain ratings 2.8 ± 0.3, $F(1,24)=107.9$, $p<0.001$] (Figure 2), consistent with the induction of a mild burn. The sensitization to heat was slightly greater at the reference site than at the two pretreated sites [Site x Time interaction for the heat pain threshold, $F(2,23)=4.03$, $p<0.05$; Site x Time interaction for heat pain ratings, $F(2,23)=5.57$, $p<0.05$]. Investigation of these interactions indicated that decreases in the heat pain threshold after the burn injury were smaller at the guanethidine site than the reference site [1.1 ± 0.4°C versus 2.0 ± 0.4°C, $F(1,24)=6.30$, $p<0.05$], but were similar at the guanethidine and saline sites

(Figure 2). The increase in heat pain ratings at the guanethidine site was similar to increases at the two control sites, indicating that the interaction was due to a greater increase in pain ratings at the reference site than the saline site [3.3 $\pm$ 0.3 versus 2.4 $\pm$ 0.4, $F(1,24)=9.31$, $p<0.01$].

As shown in Figure 3, pain ratings to electrical stimulation were lower at the heat-sensitized guanethidine site than at the two control sites, although the strength of this effect varied across the four minutes of stimulation [Site x Time interaction, $F(3.3, 49.1)=2.74$, $p<0.05$]. Ratings were significantly lower at the guanethidine site than at the saline site during the first minute [$F(1,15)=5.12$, $p<0.05$], and lower than at the reference site during the third [$F(1,15)=9.00$, $p<0.01$] and fourth minutes [$F(1,15)=9.57$, $p<0.01$].

Discussion

The findings of this study can be summarized as follows. Guanethidine had a marked excitatory effect on sensitivity to heat for at least 30 minutes. One day later the guanethidine pretreatment had little effect on sensitivity to heat before, during or after the induction of a mild burn. However, the guanethidine pretreatment inhibited electrically-evoked pain in the burnt skin.

Immediate effect of the guanethidine iontophoresis

The immediate increase in sensitivity to heat appeared to be a specific effect of guanethidine, because an iontophoresis of saline for the same duration and current intensity at an adjacent control site had only a minor effect on thermal sensations. Thermal hyperalgesia persisted for at least 30 minutes after the guanethidine administration, although an increase in the heat pain threshold at 30 minutes suggests that hyperalgesia was starting to wane. One day later, thermal hyperalgesia had disappeared completely at the site of guanethidine administration. This is consistent

with the three-hour time course of adrenergic displacement after guanethidine administration (McKain et al., 1983; Lipnicki and Drummond, 2001).

Since guanethidine ejects noradrenaline from sympathetic varicosities (Chang et al., 1965), it seems likely that an efflux of noradrenaline into the skin during and after the administration of guanethidine increased sensitivity to heat. Indeed, intradermal administration of adrenergic agents by iontophoresis or injection increases thermal hyperalgesia in non-inflamed skin (Drummond, 1996; Fuchs et al., 2001). The hyperalgesia does not seem to be due to adrenergic vasoconstriction (Drummond, 1996; Fuchs et al., 2001), but could be mediated by an increased production of nociceptive mediators such as prostaglandins or nerve growth factor (Levine et al., 1986; Gonzales et al., 1989; Tuttle et al., 1993) or might even involve direct excitation of adrenergic receptors on the peripheral projections of primary nociceptive afferents. Messenger RNA for α_1-adrenoceptors is present in the superficial dorsal horn and dorsal root ganglia of rats (Nicholson et al., 2005). Moreover, adrenergic agents such as noradrenaline and phenylephrine increase the excitability of cultured dorsal root ganglion neurons (Kasai and Mizumura, 2001; Pluteanu et al., 2002), suggesting that primary sensory afferents contain α-adrenoceptors.

Delayed effect of the guanethidine iontophoresis

Injection of adrenergic agents and stimulation of the sympathetic chain influence the discharge of nociceptive afferents in the inflamed skin of anaesthetized animals (e.g., Hu and Zhu, 1989; Sato et al., 1993; Baik et al., 2003), but normal variations in sympathetic activity generally do not exacerbate pain or hyperalgesia in the inflamed skin of healthy, awake humans (Pedersen et al., 1997; Baron et al., 1999; Elam et al., 1999; Drummond, 2001). In fact, thermal hyperalgesia in capsaicin-treated skin *decreases* during sympathetically-arousing tasks in healthy adults

(Drummond et al., 2001). Importantly, however, blockade of α-adrenergic receptors inhibits ongoing pain, nociceptive discharge and mechanical hyperalgesia in skin sensitized by an intradermal injection of capsaicin (Kinnman et al., 1997; Ren et al., 2005). Taken together, these findings suggest that inhibitory pain modulation processes override excitatory adrenergic influences on nociceptive activity during sympathetic vasoconstrictor discharge, except in the presence of a powerful mediator of nociceptor sensitization and neurogenic inflammation such as intradermal capsaicin (Kinnman et al., 1997). This might explain why depleting adrenergic stores did not affect sensitivity to heat one day after guanethidine administration in the present study, either before or after the induction of a mild burn.

It was hypothesized that local electrically-evoked release of noradrenaline from sympathetic nerve terminals would augment nociceptive sensations, and that the guanethidine pretreatment would block this effect. In support of this hypothesis, electrical stimulation of the heat-sensitized skin evoked less pain at the guanethidine-pretreated site than at the two control sites, although this varied over the four minutes of stimulation. Pain peaked during the first minute of electrical stimulation but then subsided, possibly due to adaptation of nociceptive afferents. Pain initially was greater at the saline than guanethidine site but, for unknown reasons, pain then subsided rapidly at the saline site. Pain also decreased more rapidly at the guanethidine-treated site than the reference site over the four minutes of stimulation. The guanethidine apparently did not inhibit nociceptive discharge directly, because thermal hyperalgesia was equivalent at the guanethidine and control sites.

One explanation for these findings is that noradrenaline, released locally from sympathetic varicosities during electrical stimulation, augmented electrically-evoked pain in inflamed skin. The sensations evoked by the electrical current were variously

described as “pricking”, “stinging”, “sharp”, or “burning”, implying that the current excited Aδ and C nociceptive afferents (Schady et al., 1983; Ochoa and Torebjörk, 1989). Excitation of nociceptive afferents by electrical currents provokes cutaneous vasodilatation and neurogenic inflammation (Drummond and Lipnicki, 1999; Magerl et al., 1987; Chahl, 1988) which, in turn, may disrupt the blood-nerve barrier (Antonijevic et al., 1995). Indeed, disruption of the blood-nerve barrier during capsaicin- or heat-evoked inflammation may facilitate thermal hyperalgesia and axon-reflex vasodilatation to noradrenaline (Drummond, 1998b; Houghton et al., 2006). Further studies (e.g., employing microdialysis fibres) could help to clarify whether electrical stimulation of the skin induces the local release of noradrenaline, and whether electrically-evoked neurogenic inflammation augments nociceptive sensations and axon-reflex vasodilatation to noradrenaline.

If electrical currents excite local nociceptive and sympathetic nerve fibres, it might be expected that hyperalgesia would generally develop during transcutaneous iontophoresis. In fact, heat pain ratings to a 47^{o}C stimulus were slightly greater at the saline site than in untreated skin after the 30-minute saline iontophoresis in the present study; however, the iontophoresis had little effect on heat pain thresholds. The electrical current was delivered at 32 μA/cm^2 during the 30-minute iontophoresis. A greater current density or concurrent inflammation might increase sensitivity to heat, because heat pain thresholds were lower at a site of saline iontophoresis than at a reference site in capsaicin-inflamed skin following electrical stimulation at 99 μA/cm^2 for 1 minute (Drummond, 1999) or 10 minutes (Drummond, 1998a).

Unlike other forms of neural stimulation, electrical currents can act proximal to nerve terminals. After being taken up into the cytoplasm, guanethidine inhibits depolarization by opening potassium channels in noradrenergic neurons (Fabiani and

Story, 1996). Although the effects of guanethidine probably are greatest at adrenergic nerve terminals, guanethidine might also affect neural activity more proximally due to diffusion within the cytoplasm. Further studies are required to determine whether guanethidine blocks release of noradrenaline within nerve fascicles. From an anatomical perspective, the close proximity of adrenergic and nociceptive fibres within nerve fascicles might assist their interaction during inflammation.

Clinical implications

The present findings indicate that iontophoresis of guanethidine initially provoked substantial adrenergic thermal hyperalgesia, followed by loss of thermal hyperalgesia and a reduction in electrically-evoked nociceptive sensations when stores of noradrenaline were depleted. In a subgroup of patients with complex regional pain syndrome (CRPS), pain and hyperalgesia decrease after sympathetic blockade of the affected limb (Price et al., 1998), and the pain can be rekindled by intradermal injection of adrenergic agonists (Davis et al., 1991; Torebjork et al., 1995; Ali et al., 2000). Moreover, spontaneous pain and the cutaneous distribution of mechanical dynamic and punctate hyperalgesia increase during body cooling (a strong stimulus for increased sympathetic vasoconstrictor activity) (Baron et al., 2002), presumably due to an interaction between sympathetic adrenergic neurons and nociceptive afferents in the skin. In such patients, sympathetic blockade usually prevents increases in pain during sympathetic arousal (Drummond and Finch, 2004). Further investigation of the effects of guanethidine on nociceptive sensations may help to clarify the mechanism of adrenergic hyperalgesia both in normal individuals and in patients with neuropathic pain syndromes such as CRPS.

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References

Ali Z, Ringkamp M, Hartke TV, Chien HF, Flavahan NA, Campbell JN, Meyer RA. Uninjured C-fiber nociceptors develop spontaneous activity and alpha-adrenergic sensitivity following L6 spinal nerve ligation in monkey. J Neurophysiol 1999; 81: 455-66.

Ali Z, Raja SN, Wesselmann U, Fuchs PN, Meyer RA, Campbell JN. Intradermal injection of norepinephrine evokes pain in patients with sympathetically maintained pain. Pain 2000; 88: 161-8.

Antonijevic I, Mousa SA, Schafer M, Stein C. Perineurial defect and peripheral opioid analgesia in inflammation. J Neurosci 1995; 15: 165-72.

Baik E, Chung JM, Chung K. Peripheral norepinephrine exacerbates neuritis-induced hyperalgesia. J Pain 2003; 4: 212-21.

Baron R, Wasner G, Borgstedt R, Hastedt E, Schulte H, Binder A, Kopper F, Rowbotham M, Levine JD, Fields HL. Effect of sympathetic activity on capsaicin-evoked pain, hyperalgesia, and vasodilatation. Neurology 1999; 52: 923-32.

Baron R, Schattschneider J, Binder A, Siebrecht D, Wasner G. Relation between sympathetic vasoconstrictor activity and pain and hyperalgesia in complex regional pain syndromes: a case-control study. Lancet 2002; 359: 1655-60.

Burchiel KJ. Spontaneous impulse generation in normal and denervated dorsal root ganglia: sensitivity to alpha-adrenergic stimulation and hypoxia. Exp Neurol 1984; 85: 257-72.

Chahl LA. Antidromic vasodilatation and neurogenic inflammation. Pharmacol Ther 1988; 37: 275-300.

Chang CC, Costa E, Brodie BB. Interaction of guanethidine with adrenergic neurons. J Pharmacol Exp Ther 1965; 147: 303-12.

Davis KD, Treede RD, Raja SN, Meyer RA, Campbell JN. Topical application of clonidine relieves hyperalgesia in patients with sympathetically maintained pain. Pain 1991; 47: 309-17.

Drummond PD. Noradrenaline increases hyperalgesia to heat in skin sensitized by capsaicin. Pain 1995; 60: 311-5.

Drummond PD. Independent effects of ischaemia and noradrenaline on thermal hyperalgesia in capsaicin-treated skin. Pain 1996; 67: 129-33.

Drummond PD. The effect of noradrenaline, angiotensin II and vasopressin on blood flow and sensitivity to heat in capsaicin-treated skin. Clin Auton Res 1998a; 8: 87-93.

Drummond PD. Enhancement of thermal hyperalgesia by alpha-adrenoceptors in capsaicin-treated skin. J Auton Nerv Syst 1998b; 69: 96-102.

Drummond PD. Nitroprusside inhibits thermal hyperalgesia induced by noradrenaline in capsaicin-treated skin. Pain 1999; 80: 405-12.

Drummond PD. The effect of sympathetic activity on thermal hyperalgesia in capsaicin-treated skin during body cooling and warming. Eur J Pain 2001; 5: 59-67.

Drummond PD, Finch PM. Persistence of pain induced by startle and forehead cooling after sympathetic blockade in patients with complex regional pain syndrome. J Neurol Neurosurg Psychiatry 2004; 75: 98-102.

Drummond PD, Finch PM, Skipworth S, Blockey P. Pain increases during sympathetic arousal in patients with complex regional pain syndrome. Neurology 2001; 57: 1296-303.

Drummond PD, Lipnicki DM. Noradrenaline provokes axon reflex hyperaemia in the skin of the human forearm. J Auton Nerv Syst 1999; 77: 39-44.

Elam M, Olausson B, Skarphedinsson JO, Wallin BG. Does sympathetic nerve discharge affect the firing of polymodal C-fibre afferents in humans? Brain 1999; 122 (Pt 12): 2237-44.

Fabiani ME, Story DF. Inhibition of sympathetic noradrenergic transmission by guanabenz and guanethidine in rat isolated mesenteric artery: involvement of neuronal potassium channels. Pharmacol Res 1996; 33: 171-80.

Fuchs PN, Meyer RA, Raja SN. Heat, but not mechanical hyperalgesia, following adrenergic injections in normal human skin. Pain 2001; 90: 15-23.

Gonzales R, Goldyne ME, Taiwo YO, Levine JD. Production of hyperalgesic prostaglandins by sympathetic postganglionic neurons. J Neurochem 1989; 53: 1595-8.

Houghton BL, Meendering JR, Wong BJ, Minson CT. Nitric oxide and noradrenaline contribute to the temperature threshold of the axon reflex response to gradual local heating in human skin. J Physiol 2006; 572: 811-20.

Hu SJ, Zhu J. Sympathetic facilitation of sustained discharges of polymodal nociceptors. Pain 1989; 38: 85-90.

Kasai M, Mizumura K. Increase in spontaneous action potentials and sensitivity in response to norepinephrine in dorsal root ganglion neurons of adjuvant inflamed rats. Neurosci Res 2001; 39: 109-13.

Kinnman E, Nygards EB, Hansson P. Peripheral alpha-adrenoreceptors are involved in the development of capsaicin induced ongoing and stimulus evoked pain in humans. Pain 1997; 69: 79-85.

Levine JD, Taiwo YO, Collins SD, Tam JK. Noradrenaline hyperalgesia is mediated through interaction with sympathetic postganglionic neurone terminals rather than activation of primary afferent nociceptors. Nature 1986; 323: 158-60.

Lipnicki DM, Drummond PD. Vascular and nociceptive effects of localized prolonged sympathetic blockade in human skin. Auton Neurosci 2001; 88: 86-93.

Magerl W, Szolcsanyi J, Westerman RA, Handwerker HO. Laser Doppler measurements of skin vasodilation elicited by percutaneous electrical stimulation of nociceptors in humans. Neurosci Lett 1987; 82: 349-54.

McKain CW, Urban BJ, Goldner JL. The effects of intravenous regional guanethidine and reserpine. A controlled study. J Bone Joint Surg Am 1983; 65: 808-11.

Nicholson R, Dixon AK, Spanswick D, Lee K. Noradrenergic receptor mRNA expression in adult rat superficial dorsal horn and dorsal root ganglion neurons. Neurosci Lett 2005; 380: 316-21.

Ochoa J, Torebjork E. Sensations evoked by intraneural microstimulation of C nociceptor fibres in human skin nerves. J Physiol 1989; 415: 583-99.

Pedersen JL, Rung GW, Kehlet H. Effect of sympathetic nerve block on acute inflammatory pain and hyperalgesia. Anesthesiology 1997; 86: 293-301.

Pluteanu F, Ristoiu V, Flonta ML, Reid G. Alpha(1)-adrenoceptor-mediated depolarization and beta-mediated hyperpolarization in cultured rat dorsal root ganglion neurones. Neurosci Lett 2002; 329: 277-80.

Price DD, Long S, Wilsey B, Rafii A. Analysis of peak magnitude and duration of analgesia produced by local anesthetics injected into sympathetic ganglia of complex regional pain syndrome patients. Clin J Pain 1998; 14: 216-26.

Ren Y, Zou X, Fang L, Lin Q. Sympathetic modulation of activity in Adelta- and C-primary nociceptive afferents after intradermal injection of capsaicin in rats. J Neurophysiol 2005; 93: 365-77.

Sato J, Perl ER. Adrenergic excitation of cutaneous pain receptors induced by peripheral nerve injury. Science 1991; 251: 1608-10.

Sato J, Suzuki S, Iseki T, Kumazawa T. Adrenergic excitation of cutaneous nociceptors in chronically inflamed rats. Neurosci Lett 1993; 164: 225-8.

Schady WJ, Torebjork HE, Ochoa JL. Peripheral projections of nerve fibres in the human median nerve. Brain Res 1983; 277: 249-61.

Smith AD. Mechanisms involved in the release of noradrenaline from sympathetic nerves. Br Med Bull 1973; 29: 123-9.

Torebjork E, Wahren L, Wallin G, Hallin R, Koltzenburg M. Noradrenaline-evoked pain in neuralgia. Pain 1995; 63: 11-20.

Tuttle JB, Etheridge R, Creedon DJ. Receptor-mediated stimulation and inhibition of nerve growth factor secretion by vascular smooth muscle. Exp Cell Res 1993; 208: 350-61.

Vasey MW, Thayer JF. The continuing problem of false positives in repeated measures ANOVA in psychophysiology: a multivariate solution. Psychophysiology 1987; 24: 479-86.

Zhang JM, Song XJ, LaMotte RH. An in vitro study of ectopic discharge generation and adrenergic sensitivity in the intact, nerve-injured rat dorsal root ganglion. Pain 1997; 72: 51-7.

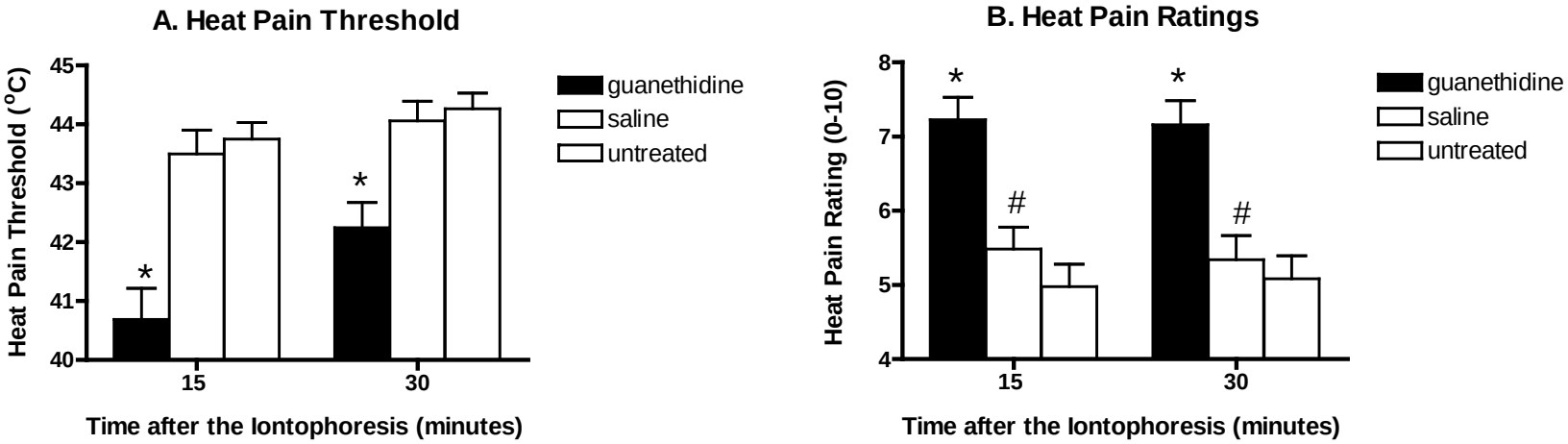
Figure legends

Figure 1. Sensitivity to heat at sites of guanethidine iontophoresis (black bars), saline iontophoresis (gray bars), and in untreated skin (clear bars) 15 and 30 minutes after the iontophoreses (N = 35). A. The heat pain threshold was lower at the site of guanethidine iontophoresis than at the other two sites (* $p<0.001$). B. Heat pain ratings to a 7-s 47^{o}C stimulus were greater at the site of guanethidine iontophoresis than at the other two sites (* $p<0.001$). In addition, heat pain ratings were greater at the saline site than the untreated site (# $p<0.05$). In Figures 1-3, error bars represent standard errors.

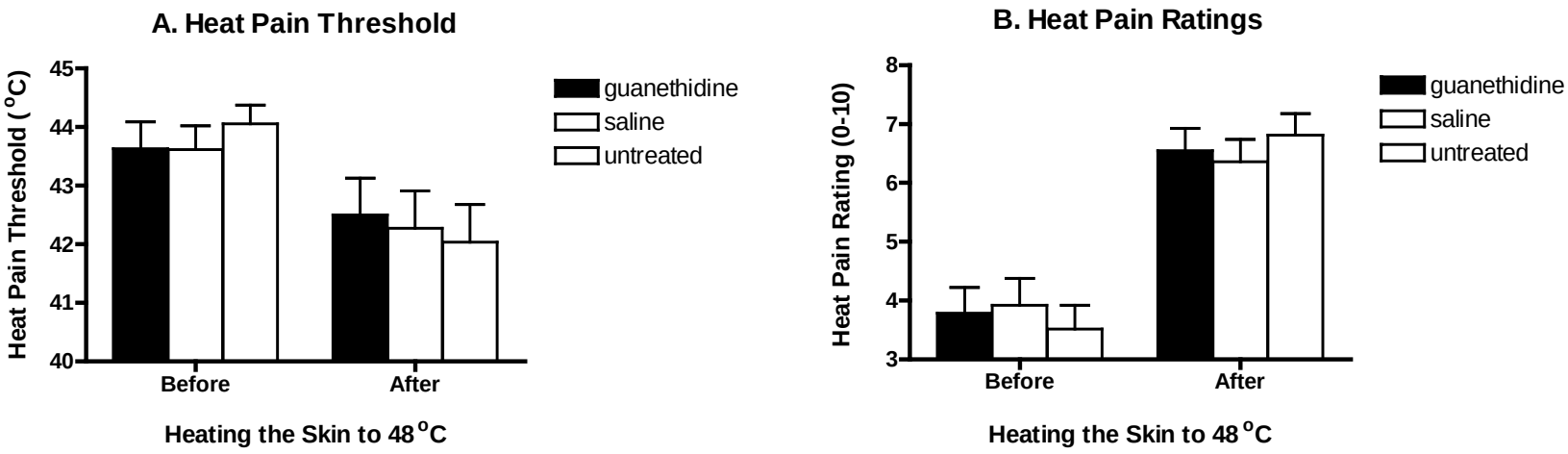
Figure 2. Sensitivity to heat 24 hours after the iontophoreses at the guanethidine (black bars) and saline sites (gray bars) and in untreated skin (clear bars), before and 30 minutes after heating each site to 48^{o}C (N = 25). A. The heat pain threshold did not differ significantly among the sites, either before or after inducing a mild burn with a 48^{o}C stimulus. However, the decrease in the heat pain threshold after the burn injury was smaller at the guanethidine site than in untreated skin ($p<0.05$). B. Heat pain ratings to a 7-s 45^{o}C stimulus did not differ significantly among the sites, either before or after the 48^{o}C stimulus. The increase in heat pain ratings at the guanethidine site was similar to increases at the other two sites. However, increases were greater at the untreated site than the saline site ($p<0.01$).

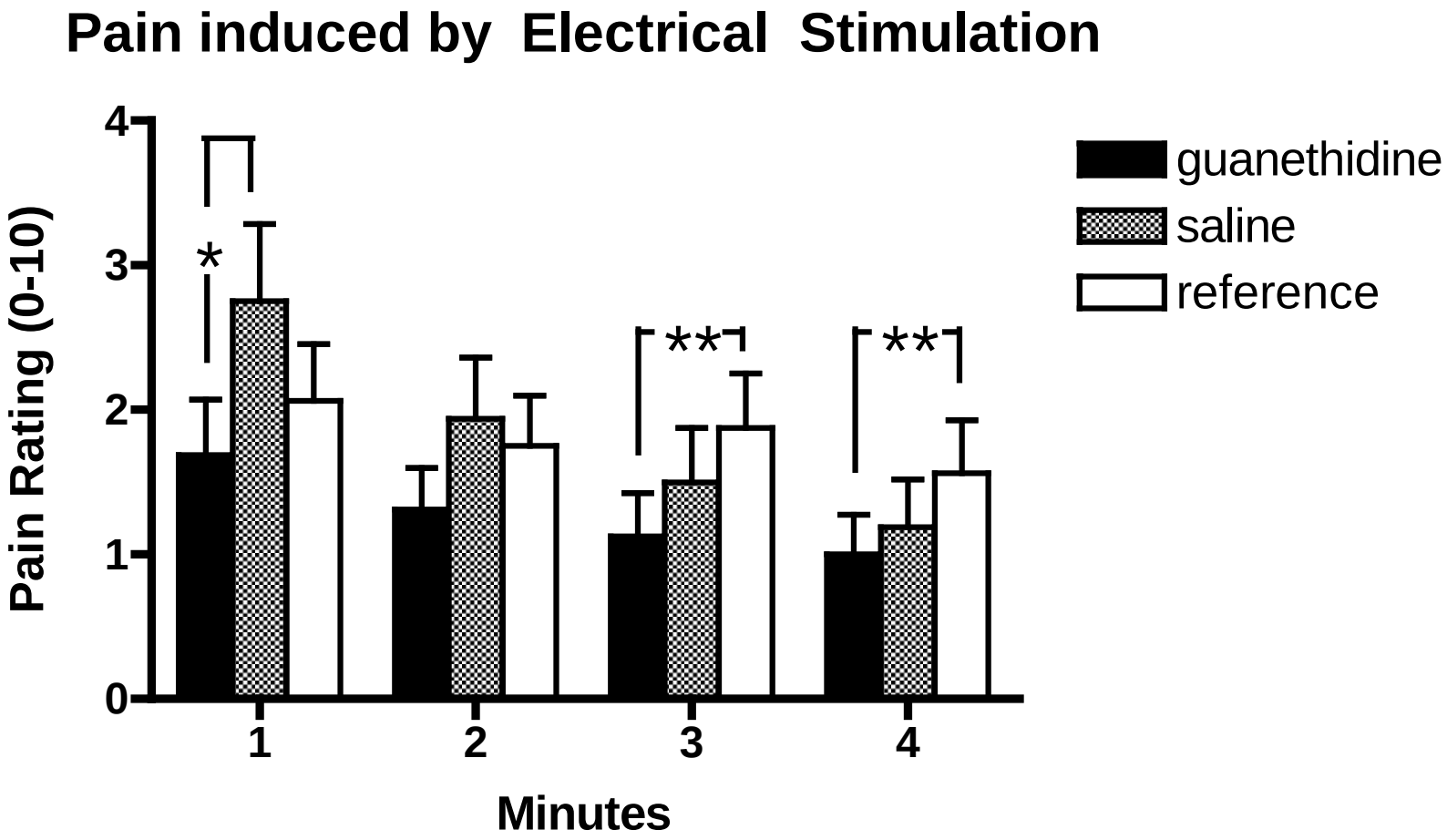
Figure 3. Mean pain ratings to four minutes of electrical stimulation at the heat-sensitized guanethidine (black bars), saline (gray bars) and untreated sites (clear bars) (N = 16). Pain ratings were significantly lower at the guanethidine site than at the saline site during the first minute (* $p<0.05$), and lower than at the untreated reference site during the third and fourth minutes (** $p<0.01$).

Immediate Effect of the Guanethidine Iontophoresis



Delayed Effect of the Guanethidine Iontophoresis




Pain induced by Electrical Stimulation
Pain Rating (0-10)
0
1
2
3
4
*
**
**
1
2
3
4
Minutes
guanethidine
saline
reference