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Involvement of opioid receptors and α_2-adrenoceptors in inhibitory pain modulation processes: a double-blind placebo-controlled crossover study

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Abstract

In healthy humans, high-frequency electrical stimulation (HFS) of the forearm not only evokes local signs of central sensitization but also triggers broader ipsilateral inhibitory influences on pain akin to a lateralized form of conditioned pain modulation. Paradoxically, some of these inhibitory influences are augmented by α_2-adrenoceptor blockade. To determine whether opioid peptides mediate inhibitory effects after HFS, the opioid receptor antagonist naltrexone was co-administered orally with the α_2-adrenoceptor antagonist yohimbine in 16 healthy women in a double-blind placebo-controlled crossover study. In each session, mechanical sensitivity in the forearms and forehead was assessed before and after HFS. In addition, pain ratings to electrical stimulation of HFS-treated or control sites in the forearm were assessed during and after painful stimulation of each temple. Unlike yohimbine alone, the naltrexone + yohimbine combination blocked analgesia evoked by HFS in the ipsilateral forehead to blunt pressure, and opposed the ipsilateral inhibitory effect of pain in the temple on electrically-evoked pain at the HFS-treated site in the forearm. These findings imply involvement of opioid peptides in an ipsilateral analgesic response that complements the more generalized form of conditioned pain modulation. Opioid mediation of this ipsilateral analgesic response appears to override opposing α_2-adrenoceptor effects.

Perspective: HFS not only evokes local signs of central sensitization but also triggers a broader ipsilateral anti-nociceptive mechanism mediated by opioid receptors. Dysfunction of this lateralized pain modulation process might contribute to painful unilateral disorders such as migraine or complex regional pain syndrome.

Key words: high frequency electrical stimulation; central sensitization; conditioned pain modulation; opioid receptors; α_2-adrenoceptors

Introduction

In healthy humans, high frequency electrical stimulation (HFS) of the forearm, cold-induced limb pain and heating the capsaicin-sensitised forearm not only induce pain and hyperalgesia at and around the site of stimulation but also inhibit sensitivity to blunt pressure in the forehead.[21,22,48-51] The inhibitory effect persists for up to 60 minutes and is stronger on the ipsilateral side, thus resembling a lateralized form of conditioned pain modulation.

The animal counterpart of conditioned pain modulation, diffuse noxious inhibitory controls, involves activation of descending inhibitory pathways from the subnucleus reticularis dorsalis in the caudal medulla to the dorsal horn of the spinal cord.[47] This subnucleus receives nociceptive information from all over the body, and acts to suppress all but the strongest sources of pain. Under certain conditions, this generalized response appears to be supplemented by a lateralized response involving noradrenergic projections from the locus coeruleus that drive an inhibitory spinal α_2-adrenoceptor mechanism.[41-43]

We previously investigated whether α_2-adrenoceptors might also mediate ipsilateral analgesia following HFS in humans by administering yohimbine, an α_2-adrenoceptor antagonist. However, results were mixed.[51] Yohimbine augmented the amplitude of the ipsilateral trigeminal nociceptive blink reflex following HFS, consistent with a pro-nociceptive effect of α_2-adrenoceptor blockade. Despite this, yohimbine failed to block analgesia in the ipsilateral forehead to blunt pressure after HFS. In our previous work, a conditioning stimulus (cold pain in the temple) inhibited a test stimulus (electrically-evoked pain at the HFS-treated site in the forearm) more strongly when the ipsilateral than contralateral temple was cooled,[48] an effect consistent with inhibitory coeruleospinal modulation of sensitized spinal neurons.[42,43] Paradoxically, however, yohimbine augmented the ipsilateral component of this analgesic response.[51]

One explanation for these diverse effects is that a blend of adrenergic and non-adrenergic influences contributes to pain modulation after HFS. These dual influences have been identified both in animal and human studies.[12,13,53] For example, in rats, contralateral capsaicin injection inhibited activity in spinal nociceptors, ostensibly via descending inhibitory controls.[13] The combination of naloxone (an opioid receptor antagonist) and phentolamine (an α_{1+2}-adrenoceptor antagonist) abolished this inhibitory influence, whereas either agent alone did not. Thus, multiple inhibitory influences, perhaps elicited independently, may converge on spinal projection neurons to block nociceptive neurotransmission.

In the current double-blind placebo-controlled crossover study, the aim was to determine whether combined opioid receptor and α_2-adrenoceptor blockade would abolish the ipsilateral analgesic response triggered by HFS. In particular, yohimbine and an opioid receptor antagonist, naltrexone, were co-administered orally before HFS conditioning. These agents enhance pain.[3,17,26-29,33,38,40] Thus, we hypothesised that together they would not only increase primary and secondary hyperalgesia in the forearm but would also inhibit ipsilateral analgesia to blunt pressure in the forehead after HFS. We also investigated the effect of combined opioid receptor and α_2-adrenoceptor blockade on pain ratings to electrical stimulation of the HFS-treated site in the forearm during and after painful stimulation of each temple. As opioid peptides contribute to conditioned pain modulation,[18,30,32,35,38,54] we hypothesized that opioid receptor blockade would inhibit the ipsilateral component of this response despite an opposing effect of yohimbine.[51]

Method

Participants

Males were not included in this study as co-administration of naltrexone and yohimbine can induce penile erection. Female participants were screened by an experimenter

not involved in determining the sequence of drug-placebo administration. Exclusion criteria included pregnancy, breast-feeding, chronic pain, psychiatric disorders, medical treatment for a condition that affected the heart, lungs, blood vessels, skin, liver or kidneys, or a known sensitivity to naltrexone or yohimbine. As a result of this screening, two female volunteers who took salbutamol for asthma were excluded, leaving a final sample of 16 women aged between 18 and 32 years (mean body weight ± standard deviation 59.7 ± 7.3 kg). This was considered to be the minimum number required to test the study hypotheses, based on previous studies of HFS.[19,20,25,46,48-51]

Recruitment began in February 2014 and data collection finished in July 2015. Participants provided their informed consent for the procedures, which were approved by Murdoch University's Human Research Ethics Committee.

Study design and drug administration

This study followed a double-blind, placebo-controlled crossover design. Naltrexone and yohimbine were co-administered in one session (the first session in five participants) and placebo in the other session (the first session in the other 11 participants). The drug-placebo order was assigned in no predetermined sequence before the participant arrived by medical personnel; thus, neither the experimenter nor the participant was aware of the treatment condition during the session. On the day of the experiment, the participant ate a normal breakfast and abstained from alcohol and caffeine. Effects of circadian rhythms were controlled by conducting the procedures at the same time of day in both sessions. To minimise carry-over effects, and to control for menstrual cycle influences on pain, the two sessions were separated by 28 days; however, the cycle stage varied across participants.

Naltrexone hydrochloride (50 mg) (Mallinckrodt Pharmaceuticals, Dublin, Ireland) and yohimbine (16 mg) (Pfizer Limited, Tadworth, Surrey, UK) were co-administered orally. Naltrexone is a nonselective opioid receptor antagonist that temporarily blocks endogenous

opioid activity at all three major classes of opioid receptors. Naltrexone and its active metabolite 6-beta-naltrexol have half-lives of 4 and 13 hours respectively. The 50 mg oral dose achieves its peak blood concentration within 60 minutes, and can block the effects of intravenously-administered opiate drugs for up to 24 h.[15] Oral administration of 16 mg of yohimbine reverses sedation and anti-nociceptive effects induced by the α_2-adrenoceptor agonist clonidine.[26] Absorption of orally-administered yohimbine is generally complete within an hour.[39] Despite a relatively short half-life, the cardiovascular effects of orally-administered yohimbine persist for several hours.[39] Thus, it is likely that both drugs were maximally active during the experimental period. To maintain blinding, the active drugs and the placebo (sugar pellets) were housed within capsules of identical appearance.

Procedures

The experimental procedures were similar to those described previously[51] (supplementary Table 1). Each session consisted of three stages (before drug administration, after drug administration, and after HFS) and lasted approximately 3 hours. In Stage 1, psychophysical tests were administered in the arms and forehead, and blood pressure and heart rate were measured. Stage 2 began with the co-administration of naltrexone + yohimbine or placebo. Sixty minutes later, the psychophysical tests were re-administered, and blood pressure and heart rate were reassessed. Stage 3 began 10 minutes after HFS with psychophysical tests, followed by an assessment of the effect of painful stimulation of the temples on pain to electrical stimulation of the forearm. All test procedures were conducted by one experimenter (LV), and participants sat in a comfortable armchair in a quiet room maintained at 22 ± 1^{o}C.

Before the experiment began, the ventral forearms were exfoliated gently with an abrasive soap (Solvol, WD40, Australia) to reduce skin electrical resistance. One ventral forearm area was assigned as the test site, and the equivalent ventral area in the contralateral

forearm as the control site. The laterality of the test and control sites was counterbalanced across participants. In the test arm, an area 1 cm from the Primary Site was designated the Secondary Site to assess secondary hyperalgesia (which reflects central sensitization).

Psychophysical tests. Participants reported pain or sharpness intensity using a verbal rating scale ranging from 0 ("no pain" or "not sharp") to 10 ("extremely painful" or "extremely sharp"). To investigate sensitivity to pinprick in the forearms, participants rated sharpness evoked by a sharp tip with a calibrated spring mechanism exerting a force of 40 g for 2 seconds (Neuro-pen, Owen Mumford, USA). To measure pressure-pain thresholds (PPT), an algometer (FDX, Wagner Instruments, USA) with a modified 8 mm diameter hemispheric rubber tip was applied at each forearm site or on each side of the forehead at 100 g/s until the participant reported pain.

The psychophysical tests were conducted with each stimulus being applied in runs alternating between the test and the control sites, and between the two sides of the forehead, in counter-balanced order across participants. To reduce variability in ratings, the participant initially was trained in both sessions until ratings and pressure-pain thresholds stabilised. Subsequently, each test was performed only once in each round. The exception was during baseline when measures taken at two sites on the same forearm differed by more than 20% (or 2 points on the 0-10 rating scales) or when the participant was uncertain about their perception of the initial stimulus. In such cases, the final measurement was the average of two readings.

Blood pressure and heart rate. At each measurement point, systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate were measured twice two minutes apart from the upper arm at heart level using an Omron M4 digital sphygmomanometer that detected blood pressure using the oscillometric method. The final reading was the average of the two measurements.

High-frequency electrical stimulation (HFS). A constant current stimulator (DS7A; Digitimer, Welwyn Garden City, UK) was used to generate the electrical stimuli, which were delivered via a custom-built electrode with 25 copper pins.[51] A ground plate was attached 1 cm from the conditioning electrode at a site not used for psychophysical testing. The electrical detection threshold (EDT) was determined using the method of limits for 2 ascending and 2 descending sets of single pulses (2 ms pulse width and an inter-pulse interval of 5 s). The stimulus intensity, starting at 0.1 mA, increased in steps of 0.1 mA until the participant perceived the stimulus, and then decreased in steps of 0.05 mA until the stimulus was no longer perceived. This procedure was then repeated. The EDT was defined as the geometric mean of the 4 stimulus intensity levels.

After 5 minutes, HFS conditioning was administered at the test site. This consisted of five 1-s bursts of electrical stimulation (100 Hz, 2 ms pulse width, at 10 times EDT up to a maximum of 8 mA) with a 9-s rest between each burst. The participant rated pain after each burst of stimulation, and the mean rating was calculated.

Pain ratings to electrical stimulation of the forearm during and after painful stimulation of each temple. Electrical stimuli (1 Hz and 0.5 ms pulse width) were delivered at the HFS-conditioned or control site in the forearm in 96 s runs, via the electrodes used to administer HFS, at an intensity which initially evoked a pain level of 5 on the 0-10 verbal rating scale. After 32 s of this stimulation (the test stimulus), an ice cube with an application surface area of 6 cm^2 was held against the left or right temple for 32 s (the conditioning stimulus). Participants rated electrically-evoked forearm pain every 2 s for 32 s prior to the ice being applied, during the 32-s conditioning period, and for 32 s after the ice was removed (the post conditioning period). In a separate control task, before any temple cooling, participants rated electrically-evoked forearm pain every 2 s for 96 s at the HFS and control sites. Rating changes during this task were subtracted from ratings during the temple cooling

task, to exclude changes that might be due to habituation. Test order was counterbalanced across participants and temple sides, and alternated between the test and control site in the forearms. As ice was applied to each temple twice (once to assess the effect on pain ratings in the HFS-treated forearm and once to assess the effect on pain ratings in the control forearm), several minutes rest was allowed between each application to minimise carry-over effects.

Statistical approach

Drug effects (naltrexone + yohimbine versus placebo) were investigated in 15 participants who completed both sessions using repeated-measures analyses of variance incorporating planned contrasts from before to after drug administration, and from before HFS conditioning (one hour after drug administration) to after HFS conditioning. After HFS, changes in sensitivity to sharpness and pressure-pain were compared between the two arms (test, control) at the primary and secondary sites, and pressure-pain thresholds were compared between the two sides of the forehead (ipsilateral versus contralateral to HFS).

Changes in electrically-evoked forearm pain during temple cooling were investigated in relation to Drug (versus placebo), HFS-conditioning (versus control arm) and Side Cooled (ipsilateral versus contralateral to the site of electrical stimulation in the forearm) with simple contrasts across Time (baseline versus the conditioning and post conditioning periods).

The criterion of statistical significance was $p < 0.05$. As hypotheses were tested with planned contrasts, interactions had only two levels and were investigated further with t-tests. Results are presented as the mean ± standard error.

Results

Drug side effects

Only one participant reported side effects during the placebo session (minor nausea). However, in the combined naltrexone + yohimbine session, most participants were agitated, anxious and restless and all participants reported that their hands felt cold. In addition, four

participants (25%) reported mild headache, and five participants (31%) experienced mild nausea and light-headedness. For most participants, symptoms subsided approximately 2 hours after drug administration. However, nausea intensified in two participants and ultimately resulted in vomiting (one shortly after the experiment had concluded and the other several hours afterwards). They also felt weak, shaky, and extremely lethargic, and had pale skin, hand tremors, and sharp stomach pain. These symptoms persisted for more than 6 hours after drug administration. Consequently, one of these participants did not return to complete the placebo session and her data were excluded from statistical analyses. There was little association between the two most common side effects (anxiety and cold hands) and any of the pain indices.

Autonomic activity

Before drugs were administered, blood pressure and heart rate were similar in the drug and placebo sessions (supplementary Fig. 1). One hour after administration, SBP and DBP had increased significantly in the combined naltrexone + yohimbine session but not in placebo session (SBP: main effect for Drug $F(1, 14) = 8.22$, $p = .012$, $\eta_p^2 = 0.37$, Drug x Time interaction $F(1, 14) = 29.2$, $p < .001$, $\eta_p^2 = 0.68$; DBP: main effect for Drug $F(1, 14) = 8.51$, $p = .011$, $\eta_p^2 = 0.38$, Drug x Time interaction $F(1, 14) = 5.89$, $p = .029$, $\eta_p^2 = 0.30$). SBP and DBP remained unchanged after HFS conditioning but were higher than in the placebo session.

Heart rate had decreased one hour after drug or placebo administration (main effect for Time $F(1, 14) = 11.7$, $p = .004$, $\eta_p^2 = 0.46$) (supplementary Fig. 1). Heart rate decreased further after HFS conditioning (main effect for Time $F(1, 14) = 10.4$, $p = .006$, $\eta_p^2 = 0.43$) but more so in the combined naltrexone-yohimbine session than in the placebo session (Drug x Time interaction $F(1, 14) = 6.44$, $p = .024$, $\eta_p^2 = 0.32$) (supplementary Fig. 1).

Forearm sensitivity

Electrical Detection Threshold (EDT). Mean EDT's were 0.32 ± 0.02 mA and 0.35 ± 0.03 mA in the combined naltrexone + yohimbine session and placebo session respectively. The mean EDT was lower than in a previous study[51] in which sites had been prepared with dry pumice stone rather than abrasive soap (0.40 ± 0.02 mA, $t(36) = 2.21$, $p = .034$).

Pain perception to HFS conditioning. The pain induced by HFS conditioning was similar in the combined naltrexone + yohimbine session (6.94 ± 0.34 on the 0-10 pain intensity scale) and the placebo session (6.64 ± 0.58).

Primary and secondary hyperalgesia. HFS evoked signs of minor primary and secondary hyperalgesia to sharp stimulation, relative to decreases in sharpness in the control arm, and also evoked primary hyperalgesia to blunt pressure. Co-administration of naltrexone and yohimbine had no consistent effect on primary or secondary hyperalgesia (supplementary Fig. 2 and 3).

Forehead sensitivity

The pressure-pain threshold (PPT) decreased from before to one hour after drug or placebo administration (main effect for Time, $F(1, 14) = 9.71$, $p = .008$, $\eta_p^2 = 0.41$) (Fig. 1). After HFS conditioning, the PPT increased (main effect for Time, $F(1, 14) = 12.38$, $p = .003$, $\eta_p^2 = 0.47$) but differed between the two sides of the forehead (Time x Side interaction, $F(1, 14) = 6.74$, $p = .021$, $\eta_p^2 = 0.33$) and sessions (Drug x Time interaction, $F(1, 14) = 4.98$, $p = .042$, $\eta_p^2 = 0.26$).

To clarify the source of the drug effect, changes in the PPT were investigated separately in each session. In the placebo session, the PPT increased on both sides of the forehead after HFS conditioning, particularly on the ipsilateral side (main effect for Time $F(1, 14) = 17.64$, $p = .001$, $\eta_p^2 = 0.56$, Time x Side interaction $F(1, 14) = 17.61$, $p = .001$, $\eta_p^2 = 0.43$). However, the PPT did not change after HFS conditioning in the combined naltrexone + yohimbine session. These findings indicate that co-administration of naltrexone and

yohimbine blocked analgesia to blunt pressure triggered by HFS conditioning, particularly in the ipsilateral forehead.

Pain ratings to electrical stimulation of the forearm during and after painful stimulation of the temple

In both sessions the current level required to evoke moderate pain in the HFS-conditioned arm (6.21 ± 0.64 mA) was lower than in the control arm (6.53 ± 0.7 mA) (main effect for Arm, $F(1, 14) = 5.34$, $p = .046$, $\eta_p^2 = .37$). Cold-pain ratings in the temples during cooling were similar in both sessions (6.7 ± 0.6 in the combined naltrexone + yohimbine session and 6.7 ± 0.5 in the placebo session). In the absence of noxious temple cooling, ratings of electrically-evoked pain in the forearm decreased from 4.9 ± 0.05 in the first 32 s block (equivalent to the period before temple cooling) to 4.4 ± 0.17 in the second block (equivalent to the conditioning period) ($F(1, 14) = 11.4$, $p = .005$, $\eta_p^2 = .45$) and to 4.1 ± 0.26 in the third block (equivalent to the post conditioning period) ($F(1, 14) = 10.8$, $p = .005$, $\eta_p^2 = .44$). These decreases were similar in both forearms in both sessions.

Generally, decreases in electrically-evoked pain in the forearm were greater when ice was applied to the ipsilateral than contralateral temple, particularly at the HFS-conditioned site (main effect for Side $F(1, 14) = 4.73$, $p = .047$, $\eta_p^2 = 0.25$, Side x Arm interaction $F(1, 14) = 7.42$, $p = .016$, $\eta_p^2 = 0.43$, Side x Block [baseline to conditioning period] interaction $F(1, 14) = 6.85$, $p = .020$, $\eta_p^2 = 0.33$; Side x Block [baseline to post conditioning period] interaction $F(1, 14) = 7.65$, $p = .015$, $\eta_p^2 = 0.35$) (Fig. 2). Importantly, during the 32-s conditioning period, electrically-evoked pain at the HFS-treated site decreased in the placebo session but increased in the combined naltrexone + yohimbine session (Drug x Arm x Block [baseline to conditioning period] interaction $F(1, 14) = 6.53$, $p = .023$, $\eta_p^2 = 0.32$).

To clarify the effect of naltrexone + yohimbine co-administration, decreases in pain during and after the ice application were explored further for each arm in each drug

condition. In the placebo session, electrically-evoked pain decreased more when ice was applied to the ipsilateral than contralateral temple, both at the HFS-treated site (Side x Block [baseline to post conditioning period] interaction $F(1, 14) = 5.21$, $p = .039$, $\eta_p^2 = 0.27$) and the control site (Side x Block [baseline to post conditioning period] interaction $F(1, 14) = 5.72$, $p = .031$, $\eta_p^2 = 0.29$) (Fig. 2). In contrast, in the combined naltrexone + yohimbine session, electrically-evoked pain at the HFS-treated site remained unchanged when ice was applied to the ipsilateral temple but increased when ice was applied to the contralateral temple (Side x Block [baseline to conditioning period] interaction $F(1, 15) = 7.83$, $p = .014$, $\eta_p^2 = 0.34$); this effect persisted during the post conditioning period (Side x Block [baseline to post conditioning period] interaction $F(1, 15) = 4.94$, $p = .042$, $\eta_p^2 = 0.25$) (Fig. 2). Painful stimulation of the temple had no consistent effect on electrically-evoked pain at the control site in the combined naltrexone + yohimbine session. Together, these findings suggest that the co-administration of naltrexone and yohimbine blocked the ipsilateral component of conditioned pain modulation at the HFS-treated site in the forearm during the ipsilateral conditioning period, and facilitated pain during the contralateral conditioning period.

Discussion

We used placebo-controlled combined opioid-receptor and α_2-adrenoceptor blockade to determine whether opioid receptors were involved in inhibitory pain-modulation processes triggered by HFS. Overall, our findings suggest involvement of opioid receptors in anti-nociceptive processes after HFS (Table 1), but not before HFS was administered.

Autonomic activity

One hour after drug administration blood pressure had increased ~4 mm Hg, virtually the same as increases after yohimbine alone.[51] This might have evoked baroreflex-induced hypoalgesia[4,7] which, if anything, should have masked the expected pro-nociceptive effects of the drug treatment. Nevertheless, pro-nociceptive effects were detected after HFS in the

naltrexone + yohimbine condition, indicating that any opposing blood pressure-mediated effect was minimal.

Opioid receptor blockade augments blood pressure during periods of stress (e.g., by blocking inhibitory opioid influences on brainstem adrenergic nuclei)[45] but has little influence on blood pressure under low-stress conditions.[11,31] In contrast, administration of yohimbine increases autonomic activity and symptoms such as restlessness and agitation under low-stress conditions.[51] Blocking α_2-autoreceptors increases the basal firing rate of neurons in brainstem adrenergic nuclei and boosts the release of adrenergic neurotransmitters from central and peripheral nerve terminals and somato-dendritic sites.[16] Hence, central and/or peripheral α_2-adrenoceptor blockade probably mediated increases in autonomic activity in this study.

Sensitivity in the forearm

HFS at 10 or 20 times the individual EDT generally triggers primary and secondary hyperalgesia.[20,36,48] The presence of only minor primary and secondary hyperalgesia after HFS in the present study might have been due to the comparatively low EDT (and hence HFS intensity which was administered at ten times the EDT). We used an abrasive soap to exfoliate the skin. This was not painful but the soap may have removed skin oils, thereby minimising skin impedance and lowering the EDT. Neither yohimbine alone in our past work[51] nor co-administration of naltrexone and yohimbine in the present study influenced pain evoked by HFS or sensitivity to mechanical stimulation of the forearms before or after HFS. Primary and secondary hyperalgesia are thought to reflect sensitization of primary afferent nociceptors and spinal wide dynamic range neurons. This sensitization is modulated by inhibitory opioid and adrenergic influences.[31,33] However, our findings suggest that descending inhibitory pain controls were inactive when participants rested quietly, as co-administration of yohimbine and naltrexone did not alter primary or secondary hyperalgesia.

Alternatively, peripheral and spinal concentrations of yohimbine and naltrexone might not have been high enough to block opioid receptors or α_2-adrenoceptors involved in modulating spinal nociceptive neurotransmission.

Analgesia to blunt pressure in the forehead

In the placebo session, sensitivity to blunt pressure decreased on both sides of the forehead after HFS of the forearm, with a greater reduction on the ipsilateral side. HFS appears to trigger a bilateral inhibitory pain-modulation mechanism (thereby resembling conditioned pain modulation) and an additional ipsilateral analgesic process, even in the presence of only modest hyperalgesia in the forearm.[49] Importantly, naltrexone + yohimbine co-administration blocked the analgesic effect of HFS to pressure-pain sensitivity in the forehead, suggesting involvement of opioid and/or α_2-adrenoceptors in this response.

Both opioid receptors and α_2-adrenoceptors are expressed on primary afferent nociceptors, where they play an inhibitory role.[34] It seems unlikely, however, that peripheral processes involving these receptors mediated ipsilateral analgesia in the forehead after HFS, due (i) to the degree of separation between the site of stimulation (the forearm) and analgesia (the forehead); and (ii) the laterality of the effect. Opioid peptides exert anti-nociceptive effects in the dorsal horn, rostroventral medulla and higher centres, and regulate descending anti-nociceptive pathways in the spinal cord.[32] Opioid and α_2-adrenoceptors are expressed widely within the central nervous system, with sites of convergence in the dorsal horn, brainstem adrenergic nuclei and the midbrain peri-aqueductal grey.[1,24,45] Opioids reduce nociceptive neurotransmission, in part, by disinhibition of brainstem noradrenergic neurons that project to the spinal cord; in turn, anti-nociceptive effects are mediated by spinal α_2-adrenoceptors on primary nociceptive afferents and second-order projection neurons.[32] Numerous animal and human studies have demonstrated synergistic interaction between opioid and adrenergic pain modulation processes exemplified, for example, by the

effectiveness of tapentadol, a combined μ-opioid receptor agonist and noradrenaline reuptake inhibitor.[6,37,44] However, our findings suggest that analgesia to blunt pressure in the forehead was mediated primarily by opioid receptors as, in our previous work, yohimbine alone was ineffective.[51]

Pain ratings to electrical stimulation of the forearm during and after painful stimulation of the temple

In our previous studies, cold-pain in the temple inhibited electrically-evoked forearm pain at a HFS-conditioned site.[48,51] Furthermore, pain reduction in the forearm was greater during ipsilateral than contralateral temple cooling, indicating the presence of an ipsilateral inhibitory pain-modulation process akin to a lateralized form of conditioned pain modulation. We observed a similar effect in the placebo session of the present study but not in the combined naltrexone + yohimbine session.

In our past work, yohimbine *facilitated* the ipsilateral component of this analgesic response after HFS, possibly by strengthening descending inhibitory controls.[51] However, the present findings indicate that additional opioid receptor blockade masked the analgesic response, thus supporting the view that opioid peptides play a primary role not only in the generalized form of conditioned pain modulation[18,30,32,35,38,54] but also in the lateralized type.

Methodological Considerations

Methodological differences, including doses and routes of administration, must be considered when comparing the present findings with those of other studies. We used a single low dose of yohimbine to minimise nonspecific effects (mediated, for example, by actions on serotonergic, dopaminergic or α_1-adrenergic receptors),[14] combined with a dose of naltrexone sufficient to block the effects of opiate drugs.[15] Thus, we cannot rule out possible involvement of non-opioid or α_2-adrenoceptor processes in mediating anti-nociceptive effects in our experimental model. Nonetheless, co-administration of yohimbine and naltrexone

blocked certain forms of analgesia triggered by HFS whereas yohimbine alone did not,[51] indicating a predominant role of the opioid system in mediating these effects.

Yohimbine and naltrexone were administered together, to determine whether naltrexone would block the facilitatory effects of yohimbine on conditioned pain modulation noted in our past work.[51] Our findings confirmed that opioid peptides are involved in conditioned pain modulation; nevertheless, it is important to investigate effects of naltrexone alone in our experimental model, to determine whether opioid receptors act independently of α_2-adrenoceptors to modify pain.

As drugs were administered orally, variation in active concentrations over the course of the study or from one participant to another might have increased variation in responses. The oral route of administration was chosen over the intravenous route to circumvent recruitment difficulties. However, pharmacodynamic interactions between yohimbine and naltrexone might have influenced the absorption or metabolism of these drugs. Dose-response studies involving intravenous administration of drugs would be required to clarify this. Nevertheless, the findings suggest that drug levels after oral administration were high enough to alter physiological activity, and that naltrexone modified the effects of yohimbine on nociceptive processing.

Although drugs were administered double-blind, it was not always possible to maintain blinding due to strong drug-induced side-effects such as nausea, agitation and headaches. These side effects might have interfered with the participants' capacity to accurately report pain thresholds and sharpness ratings. However, we are confident that drug effects were real because they included influences not only on psychophysical measures but also on conditioned pain modulation. Furthermore, drug effects were limited to the HFS-conditioned side, suggesting that effects were specific.

As our sample was small, some effects of combined opioid and α_2-adrenoceptor blockade may have been overlooked due to insufficient statistical power. However, the repeated-measures design enabled participants to act as their own control and thus compensated, at least in part, for the small sample size. We have consistently detected HFS-induced ipsilateral analgesia in mixed gender, healthy populations.[21,22,48-51] Still, it is important to determine whether disparities in adrenergic or opioid neurotransmission contribute to gender differences in pain perception in this experimental model as only females were included in this study.

Finally, certain components of the opioid and adrenergic systems might not have been active as most assessments were carried out under resting conditions (perhaps explaining why nociceptive effects of naltrexone and yohimbine co-administration were detected only after HFS). As inhibitory opioid effects on pain are stronger under stressful or painful than resting conditions,[9,10] it would be interesting to investigate effects of psychological stress on the opioid component of HFS-induced ipsilateral analgesia.

Conclusions and clinical implications

Overall, we envisage activation of ipsilateral pain-inhibitory pathways by HFS, and that supraspinal and/or spinal endogenous opioid peptides contribute to this response (Fig. 3). Conditioned pain modulation is compromised in many chronic pain syndromes, indicative of impaired descending inhibitory controls and/or up-regulation of facilitatory controls.[2,55] It is important to establish whether acute or chronic failure of the lateralised pain modulation processes explored in this study underlies symptoms in unilateral disorders such as migraine[5] or complex regional pain syndrome.[8,23]

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Table legends

Supplementary Table 1. Experimental procedure

Table 1. Expected and observed effects of HFS in the placebo and naltrexone + yohimbine sessions

Figure legends

Supplementary Fig. 1. Mean ± S.E. for (a) systolic blood pressure (SBP); (b) diastolic blood pressure (DBP); and (c) heart rate at baseline, after drug administration and after HFS conditioning in the yohimbine + naltrexone and placebo sessions. Blood pressure increased after co-administration of yohimbine and naltrexone (# $p < .05$) but did not change after placebo administration. Heart rate remained stable after co-administration of yohimbine and naltrexone but fell after administration of placebo (# $p < .05$). Blood pressure remained stable after HFS in both sessions, but heart rate fell after HFS in the yohimbine + naltrexone session (# $p < .05$). Blood pressure and heart rate were greater after drug administration in the yohimbine + naltrexone session than in the placebo session (* $p < .05$).

Supplementary Fig. 2. Mean sharpness ratings ± S.E. to stimulation of the forearm with a pin at baseline, after drug administration and after HFS conditioning. Sharpness evoked by pinprick remained stable in the test arm after HFS in both sessions, but decreased in the control arm (Time x Arm interaction $F(1, 14) = 22.5$, $p < .001$, $\eta_p^2 = .62$) (# $p < .05$). Co-administration of yohimbine and naltrexone did not influence ratings.

Supplementary Fig. 3. Mean PPT ± S.E. in the forearm at baseline, after drug administration and after HFS. The PPT decreased after HFS during both sessions in the test arm, particularly at the primary site, but remained stable in the control arm (Time x Arm interaction $F(1, 14) = 4.78$, $p = .046$, $\eta_p^2 = .26$, Time x Arm x Site interaction $F(1, 14) = 4.52$, $p = .052$, $\eta_p^2 = .24$). Co-administration of yohimbine and naltrexone did not influence the PPT.

Fig. 1. Mean PPT ± S.E. in the ipsilateral and contralateral forehead at baseline, after drug administration, and after HFS conditioning. The PPT increased on both sides of the forehead after HFS in the placebo session (# $p < .05$) but did not change after HFS in the naltrexone + yohimbine session. In the placebo session, the PPT was higher on the ipsilateral than contralateral side of the forehead after HFS (* $p < .05$).

Fig. 2. Pain ratings ± S.E. to electrical stimulation of the HFS-conditioned and control sites in the forearms during painful stimulation of the ipsilateral and contralateral temples. In the placebo session, decreases at the HFS-conditioned site were greater after conditioning the ipsilateral than contralateral temple (* $p < .05$).

Fig. 3. Schematic representation of the possible involvement of supraspinal opioid receptors and α_2-adrenoceptors in anti-nociceptive pain modulation processes.

1. Adrenergic neurons in the locus coeruleus (LC) contribute to descending inhibitory controls that inhibit neurotransmission in primary nociceptive afferents (PAN) and projection neurons (PN). These adrenergic neurons are active during periods of heightened arousal and pain, and are particularly active ipsilateral to painful stimulation of a limb.
2. Supraspinal inhibitory interneurons (IIN) modulate descending inhibitory controls.[32]
3. Opioids block activity in supraspinal inhibitory interneurons, hence releasing descending inhibitory controls.[32]
4. Yohimbine blocks inhibitory α_2-autoreceptors on brainstem adrenergic neurons, thereby augmenting descending inhibitory controls.[16,52]
5. Naltrexone restores activity in supraspinal inhibitory interneurons, thus inhibiting brainstem adrenergic neurons and blocking descending inhibitory controls.

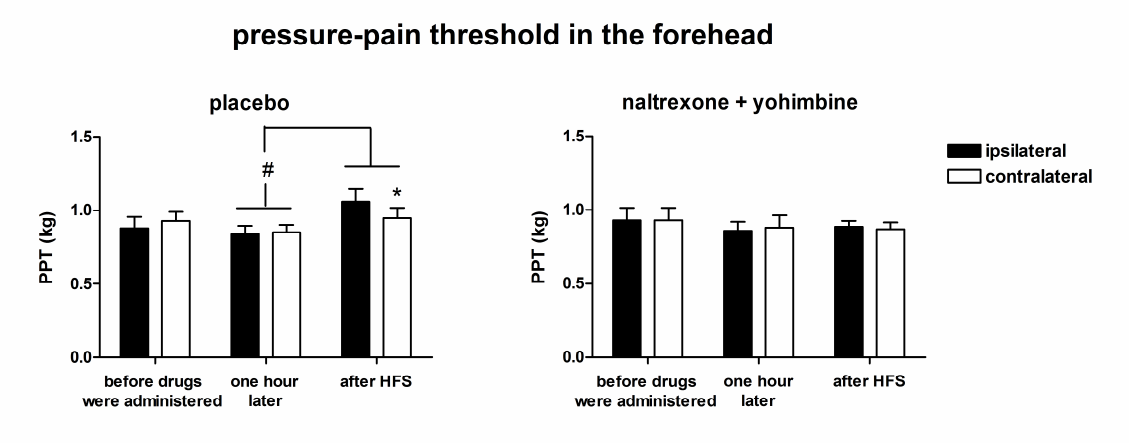
Point 4 may explain why yohimbine alone strengthened ipsilateral conditioned pain modulation in the forearm in our previous work.[51] Point 5 might explain why the co-administration of naltrexone and yohimbine blocked ipsilateral conditioned pain modulation in the forearm after HFS in the present study, and also blocked analgesia to pressure-pain in the ipsilateral forehead evoked by HFS of the forearm.

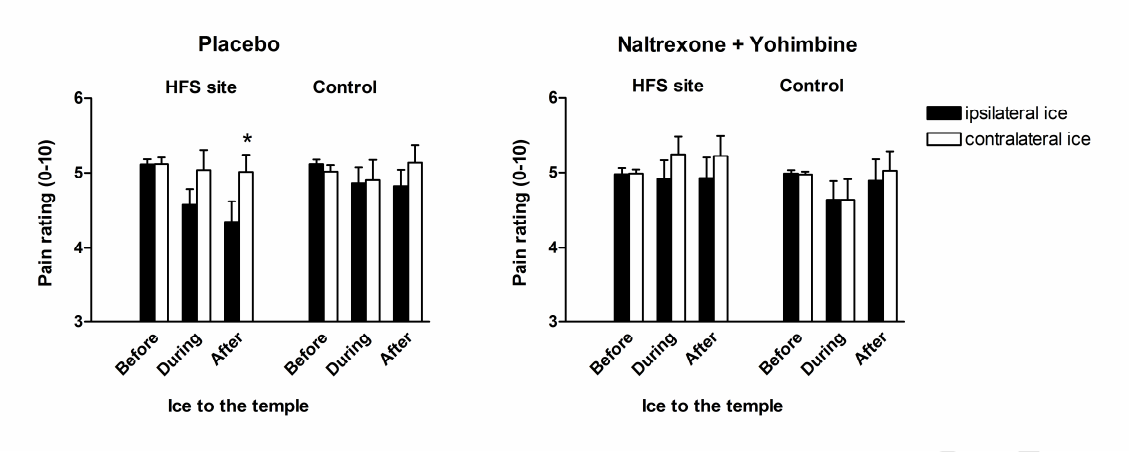
Supplementary Table 1. Experimental procedure

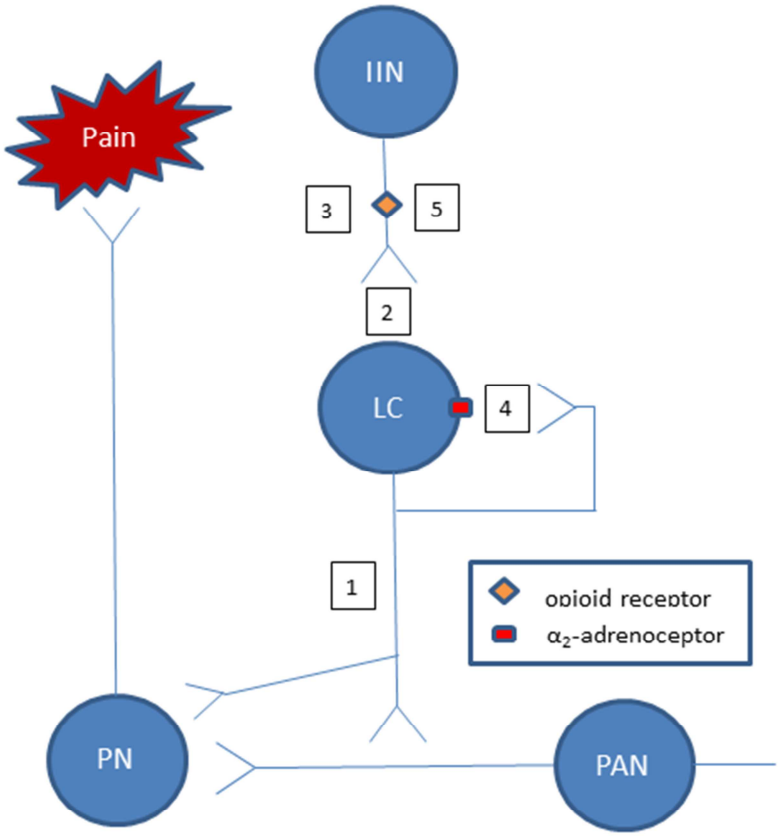
	Start Time (minutes)	Duration (minutes)	Task
Pre-drug	0	10	Psychophysical test training
	10	10	First set of psychophysical tests administered
	20	5	Blood pressure and heart rate measured twice 2 minutes apart
Post-drug	25	60	Naltrexone/yohimbine or placebo administered and absorbed
	85	10	Second set of psychophysical tests administered
	95	5	Blood pressure and heart rate measured twice 2 minutes apart
	100	10	First set of blink reflexes administered (results not reported)
	110	5	Rest
	115	5	High Frequency Electrical Stimulation (HFS) administered
	120	10	Rest
Post-HFS	130	10	Third set of psychophysical tests administered
	140	5	Blood pressure and heart rate measured twice 2 minutes apart
	145	10	Second set of blink reflexes administered (results not reported)
	155	5	Rest
	160	25	Pain ratings to electrical stimulation of the forearm during and after painful stimulation of the temples

Table 1. Expected and observed effects of HFS in the placebo and naltrexone + yohimbine sessions

	Effect of HFS	
Dependent measures	Placebo session	Naltrexone + yohimbine session
Pressure-pain threshold (forearm)		
Expected effect	↓ at the primary site	↓↓ at the primary site
Observed effect	↓ trend at the primary site	No drug effect
Sharpness (forearm)		
Expected effect	↑ at primary and secondary sites	↑↑ at primary and secondary sites
Observed effect	no change in HFS arm but ↓ in control arm	No drug effect
Pressure-pain threshold (forehead)		
Expected effect	↑ greater on the ipsilateral side	↑ blocked
Observed effect	↑ greater on the ipsilateral side	↑ blocked
Pain ratings to electrical stimulation of the forearm during and after painful stimulation of each temple		
Expected effect	↓ at the HFS-treated site greater during and after painful stimulation of the ipsilateral than contralateral temple	↓ blocked
Observed effect	↓ at the HFS-treated site greater after painful stimulation of the ipsilateral than contralateral temple	↓ blocked






IIN
Pain
3
5
2
LC
4
1
opioid receptor
α2-adrenoceptor
PN
PAN

Highlights

- Limb pain evokes an ipsilateral form of conditioned pain modulation
- Opioid peptides mediate this response in the painful limb and ipsilateral forehead
- These inhibitory opioid influences override opposing α_2-adrenoceptor effects
- Failure of this ipsilateral opioid response may aggravate chronic limb or head pain