Comparison of responses of ventral posterolateral and posterior complex thalamic neurons in naïve rats and rats with hindpaw inflammation: μ-opioid receptor mediated inhibitions

A.A. Abdul Aziz, D.P. Finn, R. Mason, V. Chapman*

E-Floor Medical School, School of Biomedical Sciences, University of Nottingham Medical School, Queen’s Medical Centre, Nottingham NG7 2UH, UK

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Abstract

The aim of the present study was to compare the effects of morphine on thalamic neuronal responses in naïve rats and rats with carrageenan-induced hindpaw inflammation. Multiple single unit ventral posterolateral (VPL) and posterior complex (Po) activity was recorded and mechanically- (7 g, 14 g, 21 g, 60 g and 80 g) evoked responses of VPL and Po neurones were measured in naïve rats and rats with carrageenan (100 μl, 2%)-induced hindpaw inflammation. Effects of systemic (0.5 mg kg⁻¹) and intra-thalamic (66 μM, 250 nL) morphine on neuronal responses were determined. Mechanically-evoked (60 g) nociceptive responses of VPL neurones were significantly larger in inflamed rats (29 ± 4 spikes s⁻¹) compared to naïve rats (19 ± 2 spikes s⁻¹, P < 0.05). Systemic morphine inhibited 7 g-evoked responses of VPL neurones in inflamed (24 ± 8% control, P < 0.01), but not in naïve rats (123 ± 3% control). Frank noxious-evoked responses of VPL neurones in inflamed rats were less sensitive to the effects of systemic and intra-thalamic morphine, compared to naïve rats (P < 0.05 for both). These data provide evidence for altered evoked responses of neurones at the level of VPL, but not at Po, during hindpaw inflammation and suggest that thalamic sites of action contribute to the effects of systemic morphine.

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1. Introduction

Noxious stimuli are detected by primary afferent nociceptors, finely myelinated A-fibres and unmyelinated C-fibres that transmit nociceptive inputs to the dorsal horn of the spinal cord (for review see Hunt and Mantyh, 2001). The spinal cord plays a major role in the integration and modulation of nociceptive inputs prior to messages being sent to higher brain centres (for review see Dickenson et al., 1997). A number of ascending pain pathways have been described, including the spinothalamic tract (STT) (Craig and Dostrovsky, 1999; Willis and Westlund, 1997) and the spinoparabrachial tract (Bester et al., 1997).

The STT relays nociceptive inputs to various nuclei in the thalamus. In the rat, one of the major targets of the SST is the ventral posterolateral (VPL) thalamic nucleus (Craig and Dostrovsky, 1999). VPL neurones have small contralateral receptive fields and are able to encode and discriminate sensory aspects of pain (Peschanski et al., 1983; Willis and Westlund, 1997). Indeed, VPL neurones respond to both innocuous and noxious mechanical stimuli and noxious thermal stimuli in the rat (Bordi and
Quartaroli 2000; Martin et al., 1996; Sherman et al., 1997). Furthermore, noxious stimulation of the hindpaw significantly increases the release of glutamate in the VPL of freely moving rats (Silva et al., 2001). Neurones in the posterior complex (Po) of the thalamus respond to noxious mechanical stimuli (Apkarian and Shi, 1994) and electrical tooth pulp stimulation (Shigenaga and Inoki, 1976).

The analgesic effects of the μ-opioid receptor agonist morphine are well described. μ-Opioid receptors are present in a large number of brain regions including the thalamus (Brodsky et al., 1995; Ding et al., 1996). Systemic administration of morphine inhibits noxious-evoked responses of thalamic neurones (Hill and Pepper 1978; Hill et al., 1982), including VPL neurones (Bordi and Quartaroli 2000; Yang et al., 1999) and neurones in the lateral part of the ventromedial thalamus (Monconduit et al., 2002) and Po neurones (Shigenaga and Inoki, 1976).

There is increasing evidence of an important contribution of higher brain centres, such as the thalamus, to hyperalgesic responses associated with peripheral injury. Ventrobasal thalamic neurones exhibit lowered thresholds and enhanced peripherally-evoked responses following hindpaw inflammation (Guilbaud et al., 1986, 1987) and nerve injury (Guilbaud et al., 1990). Moreover, thalamic N-methyl-D-aspartate (NMDA) receptors contribute to the development and maintenance of hyperalgesia in the carrageenan model of inflammatory pain (Abarca et al., 2000; Kolhekar et al., 1997). Systemic administration of morphine reduced noxious-evoked glutamate release in the VPL of the thalamus and mechanical hyperalgesia (Abarca et al., 2000), suggesting that μ-opioid receptors can modulate hyperalgesic responses at this level. These data suggest that the VPL, as well as other thalamic nuclei, may contribute to hyperalgesia associated with peripheral inflammation. It is unclear, however, whether responses of VPL neurones are facilitated following peripheral inflammation. The aim of the present study was to elucidate the effect of hindpaw inflammation on spontaneous and evoked responses of VPL and posterior (Po) neurones in vivo to determine whether peripheral inflammation alters activity of thalamic neurones. The second aim of this study was to determine whether hindpaw inflammation altered the inhibitory effects of systemic, versus intra-thalamic morphine, on spontaneous and evoked responses of VPL and Po neurones.

2. Materials and methods

Experiments were performed on male Sprague–Dawley rats weighing 220–280 g (n = 48 rats). Experimental procedures were carried out in accordance with the animals (Scientific Procedures) Act 1986 and IASP guidelines.

2.1. Surgical procedure

Anaesthesia was induced with 3% isoflurane in a 50% N2O:50% O2 mixture. The isoflurane level was reduced progressively and maintained at 1.5% throughout surgery to ensure a state of complete areflexia. The jugular vein was cannulated for intravenous administration of drugs and rats were mounted in a modified stereotaxic frame. Core temperature was monitored and maintained between 37 and 38 °C using homeothermic heating pad (Harvard Instruments, UK). A scalp incision was made, and a 5 mm diameter craniotomy was performed. The cortex above the thalamus was exposed, the dura mater was excised and exposed tissue kept moist with 0.9% sodium chloride. Experiments lasted for up to 5 h and physiological saline replacement was given intermittently at regular intervals (approximately 0.75–0.9 ml/h).

2.2. Recording procedure

An eight microwire electrode array (Teflon-coated stainless steel, 50 μm diameter per wire; NB Labs, Texas, USA) was used to record spike activity from a number of single neurones. Electrodes were placed in the VPL (3.2 mm posterior, 3.0–3.2 mm lateral and 5.0–7 mm ventral from bregma) or the Po (3.6 mm posterior, 1.4–2.0 mm lateral and 5.0–6.5 mm ventral from bregma) of the thalamus, according to the atlas of Paxinos and Watson (1997). The electrode assembly was clamped to a Narishighe manipulator which was used to progressively lower the array through the right thalamus until neurones that responded to brush and pinch stimulation of the contralateral hindpaw were identified. Search stimuli were applied to the contralateral hindpaw intermittently, for short periods of time (around 1–2 s), to ensure that neurones did not become sensitized.

The electrode array was connected via an eight-channel field-effect transistor unity gain headstage (HST/8m-G1, Plexon Inc, TX, USA) to a multichannel preamplifier (gain ×1000, band-pass filtered 150 Hz–9 kHz; Plexon Inc. http://www.plexoninc.com). Extracellular action potential spikes were then fed to a Plexon Multichannel Acquisition Processor Box linked to a host PC (Dell 1.5 GHz running Windows 2000), provided simultaneous 40 kHz (25 μs) A/D conversion on each channel at 12 bit resolution. The Multichannel Acquisition Processor system further provided additional programmable amplification and filtering of spikes (final gain up to ×32,000, final bandwidth 400 Hz–5 kHz). Spike discrimination (up to four spikes per channel) was achieved with pairs of voltage-time windows or principal
component analysis (Abeles and Goldstein, 1977). Specialised software (Plexon Real-time Acquisition System Programs for Unit Timing in Neuroscience-RASPUTIN) comprising a series of client/server programs running with a Windows™-based operating system provided spike sorting, data visualisation and analysis in real time.

2.3. Peripheral cutaneous stimulation

Calibrated von Frey monofilaments (7 g, 14 g, 21 g, 60 g and 80 g) were applied to the contralateral receptive field of VPL and Po neurones on the hindpaw of naıve rats and evoked responses were recorded. A pilot study demonstrated that 10 s stimulation of the receptive field with 60 and 80 g stimuli produced a sustained firing of VPL neurones, but not Po neurones. Po neurons required a longer (20 s) duration of stimulation. Thus, von Frey monofilaments were applied, in ascending order, to the peripheral receptive field of the hindpaw for 10 s for the study of VPL neurones (n = 52 neurones, 12 rats) and 20 s for the study of Po neurones (n = 36 neurones, 6 rats). Neuronal responses (spikes s⁻¹) to mechanical stimuli were recorded at 10 min intervals. Prior to any intervention, control mechanically-evoked responses of VPL and Po neurones were recorded for up to 60 min to ensure that control responses were stable. The last 3 sets of control mechanically-evoked responses of neurones were averaged to give the mean control mechanically-evoked response for subsequent studies of drug effects. The noxious withdrawal threshold in awake animals is 15 g (Chaplan et al., 1994), thus weights exceeding the 15 g threshold are described as noxious.

In separate groups of rats (VPL: n = 6; Po: n = 6), 100 μl of 2% carrageenan (Sigma) in saline was injected into the plantar surface of the left hindpaw. Mechanically-evoked (as described above) responses of contralateral VPL and Po neurones were recorded prior to, and at 10 min intervals for 2 h 30 min after intraplantar injection of carrageenan.

2.4. Drug administration

Effects of intravenous administration of morphine (0.5 mg kg⁻¹) on the mechanically-evoked responses of VPL neurones (n = 12 rats) and Po neurones (n = 6 rats) in naïve rats were studied over a period of 30 min. The ability of the opioid receptor antagonist naloxyone (0.2 mg kg⁻¹), administered 2 min after administration of morphine, to prevent the inhibitory effects of morphine on mechanically-evoked responses of VPL neurones was studied (n = 6 rats). The ability of naloxyone (0.2 mg kg⁻¹), administered 30 min after morphine, to reverse the inhibitory effects of morphine on mechanically-evoked responses of Po neurones was studied (n = 6 rats).

In carrageenan-treated rats, cumulative doses of morphine (0.5–2 mg kg⁻¹) were administered intravenously at two and a half hours following peripheral injection of carrageenan, and the effects of morphine on mechanically-evoked responses of VPL and Po neurones were studied at 10 min intervals for 30 min (n = 6 rats, for each region). The ability of naloxyone (0.2–1 mg kg⁻¹), given 30 min after morphine, to reverse the effects of morphine on mechanically-evoked responses of neurones was also studied (n = 6 carrageenan-treated rats).

For intra-thalamic injections, a 31G stainless-steel cannula was attached in parallel with the microwire array, with the cannula tip approximately 300 μm from the electrode tips. Effects of intra-thalamic injection of morphine (66 μM, 250 nL, injected over a 1.5 min period) versus vehicle on innocuous (7 g) and noxious (60 g) mechanically-evoked responses of VPL neurones were studied in naïve rats (n = 6) and carrageenan-treated rats (n = 6). The dose of morphine was selected on the basis of previous studies of the effects of intracerebral administration of morphine (Freidrich and Gebhart, 2003).

2.5. Monitoring of mean arterial blood pressure and heart rate

In a separate group of naïve rats (n = 6), the femoral artery was cannulated and mean arterial blood pressure (MAP) and heart rate were recorded using Chart 3.1 (MacLab) and Apple Mac computer. Effects of cumulative intravenous doses of morphine (0.5–2 mg kg⁻¹) on MAP and heart rate were recorded for 30 min.

2.6. Histology

At the end of each experiment, current (10 μA) was passed for 10 s through the electrode to deposit ferric ions. Rats were perfused transcardially with 0.9% NaCl followed by 4% potassium ferricyanide. Brains were removed and stored overnight in 4% paraformaldehyde. Tissue blocks were sectioned transversely at 100 μm using a vibratome (Campden Instruments, UK). Iron deposits at the electrode tips were revealed by the Prussian Blue reaction (Hong et al., 2000). Recording sites were identified with reference to the rat brain atlas of Paxinos and Watson (1997).

2.7. Data analysis

For each neurone isolated, the spontaneous activity was recorded throughout the study, the mean spontaneous activity was calculated and expressed as spikes s⁻¹. To allow comparisons between the absolute magnitudes of evoked responses of neurones, spontaneous activity (spikes s⁻¹) of neurones was measured for 5 min prior to application of mechanical stimuli and
was subtracted from mechanically-evoked responses (spikes s⁻¹) of the neurone. This ensured that any changes in ongoing spontaneous activity as a result of drug intervention did not bias mechanically-evoked responses of neurones.

Burst activity was also measured. On the basis of previous studies, a burst was defined as the occurrence of 6 spikes within any 20 ms period during 150 ms (Hartings et al., 2003) of spontaneous activity with the first interspike interval less than 6 ms and the subsequent interspike intervals less than 15 ms (Radhakrishnan et al., 1999; Tsoukatos et al., 1997). The number of bursts min⁻¹ and mean burst duration were calculated under the different experimental conditions.

Data were analysed off-line using Off-Line Sorter (Version 2.1, Plexon Inc, USA), NeuroExplorer (Version 3.1, Nex Inc, USA), and Prism (Version 3.03, GraphPad, USA). Data were non-parametric and therefore were analysed using Mann–Whitney or Wilcoxon matched pairs test when appropriate. Data are expressed as mean ± SEM (standard error of mean), statistical significance was taken when P < 0.05.

3. Results

3.1. Recordings from VPL neurons

3.1.1. Spontaneous activity and mechanically-evoked responses of VPL neurones in naïve rats and rats with hindpaw inflammation

Neurones (n = 52) recorded in naïve rats (n = 12) were histologically identified as being located within the VPL (Fig. 1). Approximately 40% of these VPL neurones (20/52 neurones) responded to peripheral mechanical stimulation, the remaining neurones did not respond to the mechanical stimuli used. There was no significant difference in the spontaneous activity of VPL neurons that responded to mechanical stimuli (Fig. 2) and those that did not respond to the mechanical stimuli used (data not shown). Stimulation of the hindpaw receptive with innocuous and noxious mechanical stimuli evoked graded increases in the firing of VPL neurones (Fig. 2). In all cases, mechanically-evoked responses were significant compared to spontaneous activity of neurones (P < 0.01, for all weights).

Neurones (n = 24) recorded in rats with carrageenan-induced hindpaw inflammation (n = 6) were histologically identified as being located within the VPL (Fig. 1). Half of the VPL neurones (12/24 neurones) responded to peripheral mechanical stimulation. There was no significant difference in the spontaneous activity of VPL neurons that responded to mechanical stimuli (Fig. 2) and those that did not respond to the mechanical stimuli used (data not shown). Mechanical stimulation of the hindpaw receptive field evoked graded increases in the firing of VPL neurones, compared to spontaneous activity, in rats with established hindpaw carrageenan-induced inflammation (Fig. 2A). In all cases, mechanically-evoked responses were significant compared to spontaneous activity of neurones (P < 0.01, for all weights). Noxious (60 and 80 g) evoked responses of VPL neurones were significantly larger in rats with hindpaw carrageenan-induced inflammation, compared to naïve rats (Fig. 2A; P < 0.05). These differences in 60
and 80 g-evoked responses of VPL neurones were not due to differences in spontaneous activity of VPL neurones of the two groups of rats (Fig. 2A).

3.1.2. Effects of morphine on spontaneous activity and mechanically-evoked responses of VPL neurones in naïve rats and rats with hindpaw inflammation

In both groups of rats, the mean spontaneous firing rate of VPL neurones was not significantly altered by morphine or naloxone, compared to pre-drug control values (data not shown). In naïve rats (n = 12), systemic administration of morphine (0.5 mg kg\(^{-1}\)) did not alter lower weight (7 and 14 g) mechanically-evoked responses of VPL neurones (n = 20 neurones, Fig. 2B). Morphine attenuated higher weight (21, 60 and 80 g) mechanically-evoked responses of VPL neurones, compared to pre-drug controls (P < 0.05 for all, Fig. 2B). Inhibitory effects of systemic morphine were maximal at 10–20 min post-administration. Pre-administration of naloxone (0.2 mg kg\(^{-1}\)) prevented the inhibitory effects of morphine on 21–80 g-evoked responses of VPL neurones (range of reversal from 98 ± 7% to 107 ± 2% of control, n = 6 rats).

In rats with hindpaw inflammation (n = 6 rats), systemic administration of morphine (0.5 mg kg\(^{-1}\)) significantly inhibited lower weight (7 and 14 g) mechanically-evoked responses of VPL neurones (n = 12 neurones), compared to pre-drug control values (P < 0.05, Fig. 2B). Inhibitory effects of morphine on 7 and 14 g-evoked responses of VPL neurones in rats with hindpaw inflammation were significant compared to the lack of effect of morphine on 7 and 14 g-evoked responses of VPL neurones in naïve rats (Fig. 2B).

Morphine also inhibited 21 g-evoked responses in rats with hindpaw inflammation compared to pre-drug controls (P < 0.05). By contrast, morphine did not significantly inhibit 60 g and 80 g-evoked responses of VPL neurones in rats with hindpaw inflammation, compared to pre-drug controls. There was a significant difference between the effects of morphine on 60 and 80 g-evoked responses of VPL neurones in naïve rats and rats with hindpaw inflammation (Fig. 2B). To determine whether this represented a shift in the effectiveness of morphine, we evaluated the effects of higher doses of morphine on mechanically-evoked responses of VPL neurones in rats with hindpaw inflammation. Higher doses of morphine (1 and 2 mg kg\(^{-1}\)) produced dose-related inhibitions of 60 g and 80 g-evoked responses of VPL neurones in rats with hindpaw inflammation (1 mg kg\(^{-1}\): 53 ± 9%, 47 ± 11% of control respectively; 2 mg kg\(^{-1}\): 19 ± 14%, 25 ± 8% of control, respectively). Effects of morphine (2 mg kg\(^{-1}\)) on 60 g and 80 g-evoked responses were significantly (P < 0.05) reversed by post-administered naloxone (1 mg kg\(^{-1}\)) to 60 ± 11% of control and 64 ± 10% of control, respectively (n = 6 rats).

Effects of intra-thalamic administration of morphine (66 μM, 250 nL) on innocuous and noxious mechanically-evoked responses of neurones were also studied in separate groups of naïve rats (n = 6 rats) and rats with hindpaw inflammation (n = 6 rats). Morphine did not significantly alter 7 g-evoked response of VPL neurones in naïve rats, but attenuated (61 ± 4% of control) 7 g-evoked responses of VPL neurones in rats with hindpaw inflammation. Inhibitory effects of intra-thalamic morphine were significant (P < 0.05) compared to the effects of intra-thalamic vehicle (80 ± 4% of control). Intra-thalamic morphine inhibited noxious-evoked responses of VPL neurones in naïve rats (21 ± 2% of control) and rats with hindpaw inflammation (56 ± 1% of control), compared to pre-drug values and the effect of vehicle (91 ± 4% of control). Intra-thalamic morphine had significantly greater inhibitory effects on noxious-evoked responses of VPL neurones in naïve rats, compared to rats with hindpaw inflammation (P < 0.05).
Table 1  
Effects of systemic administration of morphine on burst index of (A) VPL and (B) Po neurons in naïve rats (n = 6 rats) and in rats with hindpaw inflammation (n = 6)  

<table>
<thead>
<tr>
<th></th>
<th>Naïve (prior to morphine)</th>
<th>Naïve (post-morphine)</th>
<th>Naïve (prior to hindpaw inflammation)</th>
<th>Carrageenan</th>
<th>Carrageenan + morphine</th>
</tr>
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<tbody>
<tr>
<td><strong>(A) Effects of systemic morphine on burst index of VPL neurones</strong></td>
<td></td>
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<tr>
<td>Number of burst min⁻¹</td>
<td>0.66 ± 0.21</td>
<td>1.75 ± 0.16*</td>
<td>0.40 ± 0.17</td>
<td>0.58 ± 0.32</td>
<td>1.85 ± 0.53*</td>
</tr>
<tr>
<td>Mean duration of burst (s)</td>
<td>0.14 ± 0.06</td>
<td>0.05 ± 0.02</td>
<td>0.06 ± 0.02</td>
<td>0.09 ± 0.03</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td><strong>(B) Effects of systemic morphine on burst index of Po neurones</strong></td>
<td></td>
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</tr>
<tr>
<td>Number of burst min⁻¹</td>
<td>1.85 ± 0.80</td>
<td>2.71 ± 1.17</td>
<td>2.10 ± 1.15</td>
<td>1.14 ± 0.49</td>
<td>1.2 ± 1.00</td>
</tr>
<tr>
<td>Mean duration of burst (s)</td>
<td>0.09 ± 0.02</td>
<td>0.06 ± 0.02</td>
<td>0.12 ± 0.04</td>
<td>0.07 ± 0.03</td>
<td>0.06 ± 0.04</td>
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</table>

Morphine significantly increased number of bursts min⁻¹ of VPL neurones in both naïve rats and rats with hindpaw inflammation, compared to pre-drug values (*P < 0.05). Statistical comparisons between the effects of morphine with pre-drug values were performed with Wilcoxon matched pairs test.

3.2. Effects of systemic morphine on burst activity of VPL neurons

Burst activity of VPL neurones was analysed under the different experimental conditions. The burst index (number of burst min⁻¹ and mean duration of burst) of these neurones was very similar in naïve rats and rats with hindpaw inflammation (Table 1). In both naïve rats and rats with hindpaw inflammation, systemic administration of morphine significantly increased the number of burst min⁻¹ of VPL neurones, compared to pre-drug values (*P < 0.05). Statistical comparisons between the effects of morphine with pre-drug values were performed with Wilcoxon matched pairs test.

3.3. Recordings from Po neurones

3.3.1. Spontaneous activity and mechanically-evoked responses of Po neurones in naïve rats and rats with hindpaw inflammation

Neurones (n = 36) recorded in naïve rats (n = 6) were histologically identified as being located within the Po of the thalamus (Fig. 1); 14/36 neurones responded to peripheral mechanical stimulation, the remaining neurones did not respond to peripheral mechanical stimulation. Spontaneous activity of Po neurones that were responsive to peripheral mechanical stimuli (Fig. 3A) was significantly larger (P < 0.05) than spontaneous activity of non-responsive neurones (7 ± 2 spikes s⁻¹).

Following application of mechanical stimuli to the contralateral hindpaw receptive field, Po neurones exhibited graded increases in evoked responses. Mechanically-evoked responses of Po neurones were significant, compared to spontaneous activity (P < 0.05), with the exception of the 7 g-evoked response (Fig. 3A).

Neurones recorded in rats with hindpaw inflammation (n = 6 rats) were histologically identified as being located within the Po of the thalamus (Fig. 1), 12/30 neurones responded to peripheral mechanical stimuli. There were no significant differences in the spontaneous activity of Po neurones that responded to mechanical stimuli before and at 3 h after injection of carrageenan (Fig. 3A). The spontaneous activity of non-responsive Po neurones was not significantly altered by intraplantar injection of carrageenan (4 ± 0.6 spikes s⁻¹). Significant
differences in the spontaneous activity of Po neurones that respond to mechanical stimuli and non-responsive Po neurones were still present at 3 h after injection of carrageenan (P < 0.05).

Following application of mechanical stimuli to the contralateral hindpaw receptive field of rats with hindpaw inflammation, Po neurones (n = 12 in n = 6 rats) exhibited graded evoked responses. Mechanically-evoked responses of Po neurones were significant, compared to spontaneous activity (P < 0.05), with the exception of the 7 g-evoked response (Fig. 3A). Overall, there were no significant differences in the frequencies of mechanically-evoked responses of Po neurones in rats with hindpaw inflammation and naïve rats (Fig. 3A).

3.3.2. Effects of morphine on spontaneous activity and mechanically-evoked responses of Po neurones in naïve rats and rats with hindpaw inflammation

Systemic administration of morphine (0.5 mg kg$^{-1}$) did not alter the mean spontaneous activity of Po neurones in naïve rats or rats with hindpaw inflammation, compared to pre-drug control values (data not shown). Systemic administration of morphine (0.5 mg kg$^{-1}$) did not alter 7 g-evoked responses of Po neurones in naïve rats or rats with hindpaw inflammation. Morphine did, however, significantly inhibit higher weight (14, 21, 60 and 80 g) mechanically-evoked responses of Po neurones in naïve rats, compared to pre-drug controls (Fig. 3B). Inhibitory effects of morphine were maximal between 10 and 20 min post-administration. Post-administration of naloxone (0.2 mg kg$^{-1}$) significantly (P < 0.05) reversed the inhibitory effects of morphine on mechanically-evoked responses of Po neurones in naïve rats and rats with hindpaw inflammation (range of reversal for 14–80 g-evoked responses: 70 ± 1% to 80 ± 13% of control, n = 6 rats).

3.4. Effects of systemic morphine on burst activity of Po neurones

There was no significant difference in the burst index (number of burst min$^{-1}$ and mean duration of burst) of Po neurones in naïve rats and in carrageenan-treated rats (Table 1). Systemic administration of morphine did not significantly alter the burst index of Po neurones compared to pre-drug controls (Table 1).

3.5. Effects of morphine on mean arterial blood pressure and mean heart rate in anaesthetized rats

MAP in anaesthetized rats was 81 ± 2 mmHg (n = 6). Systemic administration of the lower dose of morphine (0.5 mg kg$^{-1}$) did not significantly alter MAP. Higher doses of morphine (1 and 2 mg kg$^{-1}$) decreased MAP at 1 min post-administration (67 ± 2 mmHg; 63 ± 1 mmHg, respectively). These effects of morphine were transient, 10 min after administration of morphine (1 and 2 mg kg$^{-1}$) MAP had returned to pre-drug control values (78 ± 3 mmHg and 80 ± 1 mmHg, respectively). Similarly at 20 min after administration of morphine (1 and 2 mg kg$^{-1}$) there was no difference in MAP (79 ± 1 mmHg and 80 ± 2 mmHg, respectively), compared to pre-drug control values. None of the doses of morphine significantly altered the mean heart rate, compared to pre-drug control values in anaesthetized rats (86 ± 5% to 98 ± 3% of control).

4. Discussion

4.1. Responses of VPL and Po neurones

The present study demonstrates some marked differences in the spontaneous and evoked responses of neurones in two thalamic nuclei (VPL and Po) in naïve rats and rats with hindpaw inflammation. The spontaneous activity of Po neurones responding to mechanical stimulation was significantly higher than spontaneous activity of VPL neurones that responded to these stimuli. By contrast, Po neurones that did not respond to peripheral mechanical stimulation had spontaneous activity which was similar to that of VPL neurones. In keeping with earlier work (Martin et al., 1996), systemic administration of the µ-opioid receptor agonist morphine did not significantly alter the frequency of spontaneous activity of VPL or Po neurones in naïve rats. Systemic morphine did, however, increase the number of bursts min$^{-1}$ of VPL neurones, but not Po neurones in naïve rats.

Intraplantar injection of carageenan into the hindpaw resulted in a swelling of the hindpaw, which was comparable to that previously reported (Kelly et al., 2003). At two and a half hours following injection of carrageenan, spontaneous activity of VPL and Po neurones was not significantly different to that observed in naïve rats. Similarly, burst activity of VPL and Po neurones was not altered in rats with hindpaw inflammation, compared to naïve rats. Our data are consistent with the earlier report that spontaneous activity of ventrobasal thalamic neurones is not altered by this type of hindpaw inflammation (Guilbaud et al., 1986) and observations that peripheral carrageenan-induced inflammation does not alter spontaneous activity of spinal neurones (Stanfa and Dickenson, 1993). Although administration of morphine did not alter the frequency of spontaneous activity of VPL neurones in rats with hindpaw inflammation, morphine did increase the number of bursts min$^{-1}$ of VPL neurones in a similar manner to that observed in naïve rats. Future studies will further investigate the significance of this finding.
In naïve rats, a proportion of VPL and Po neurones were responsive to peripheral mechanical stimulation of the hindpaw receptive field. Mechanically-evoked responses of VPL and Po neurones were significant compared to spontaneous activity, with the exception of the lowest weight (7 g) for Po neurones. VPL neurones responded to innocuous stimuli (7 g and 14 g) and exhibited graded increases in responses to increasing weights of noxious (> 14 g) mechanical stimuli. These data fit well with previous reports that VPL neurones respond weakly to innocuous stimuli and encode noxious stimuli in the rat (Bordi and Quartaroli, 2000; Martin et al., 1996). In the present study we did not systematically study receptive field sizes of VPL neurones, however previous studies have reported that VPL neurones have restricted contralateral receptive fields (Guilbaud et al., 1980).

Low weight-evoked responses of VPL neurones in rats with hindpaw inflammation were similar in magnitude to responses of VPL neurones in naïve rats. We have previously shown this also to be the case for low weight-evoked responses of spinal neurones (Kelly et al., 2003). By contrast, responses of VPL neurones to the higher noxious mechanical stimuli (60 g and 80 g) were significantly larger in rats with hindpaw inflammation, compared to naïve rats. We have not observed changes in noxious-evoked responses of spinal neurones following hindpaw inflammation (Kelly et al., 2003), suggesting that these facilitated responses of VPL neurones are not exclusively due to peripheral sensitization or changes at the level of the spinal cord, but may arise due to supraspinal changes in excitability. Our data support studies demonstrating that N-methyl-D-aspartate (NMDA) receptors (Kolhekar et al., 1997) and the release of glutamate in the thalamus (Abarca et al., 2000) contribute to the development and maintenance of thermal and mechanical hyperalgesia associated with hindpaw carrageenan inflammation. Nociceptive responses of ventrobasal thalamic neurones are also enhanced following acute carrageenan inflammation (Guilbaud et al., 1986). Thus, enhanced noxious-evoked responses of VPL and ventrobasal thalamic neurones are associated with this model of inflammation and may contribute to the mechanical hyperalgesia.

In the present study, Po neurones responded to higher weight (minimum of 14 g) mechanical stimuli and required a longer duration of stimulus application, compared to VPL neurones. Po neurones did not exhibit a strong encoding of mechanical stimuli and frequencies of mechanically-evoked responses of Po neurones were smaller than mechanically-evoked responses of VPL neurones. These data corroborate an early report that Po neurones do not respond to touch and pressure, but respond to pinch (Apkarian and Shi, 1994). In contrast to the VPL neurones, mechanically-evoked responses of Po neurones were not altered by hindpaw carrageenan-induced inflammation. However, potential changes in receptive field sizes were not measured in the present study and, therefore, the importance of these effects are unknown. These data indicate that the effects of carrageenan inflammation on responses of VPL neurones are not due to generalized changes, for example in the physiological state of the animal, and indicate that changes in the response properties of Po neurones are unlikely to contribute to hyperalgesia associated with hindpaw inflammation.

4.2. Effects of morphine on evoked responses of VPL and Po neurones

Systemic administration of morphine (0.5 mg kg\(^{-1}\)) inhibited high, but not low weight-evoked responses of VPL neurones in naïve rats. This finding is consistent with earlier studies demonstrating that morphine decreases the responses of nociceptive neurones in the thalamus (Hill et al., 1982), VPL (Martin et al., 1996; Yang et al., 1999), Po (Shigenaga and Inoki, 1976), thalamic nucleus submedius (Fu et al., 2002) and ventromedial thalamus (Monconduit et al., 2002, 2003). Following carrageenan-induced hindpaw inflammation, we observed marked differences in the effects of systemic morphine on the responses of VPL neurones, compared to naïve rats. Morphine (0.5 mg kg\(^{-1}\)) significantly inhibited lower weight mechanically-evoked responses of VPL neurones in rats with hindpaw inflammation, but not naïve rats. By contrast, frank noxious (60 and 80 g) evoked responses of VPL neurones of rats with hindpaw inflammation were less sensitive to morphine, compared to naïve rats. This reduced sensitivity to morphine is likely to be due to the higher discharge rate of VPL neurones of rats with hindpaw inflammation. Higher doses of morphine inhibited 60 g and 80 g-evoked responses of VPL neurones in rats with hindpaw inflammation. In contrast to the VPL neurones, mechanically-evoked responses of Po neurones in naïve rats and rats with hindpaw inflammation were equally sensitive to the low dose of systemic morphine. Inhibitory effects of systemic morphine on VPL and Po neurones were prevented, or reversed, by pre- and post-administered naloxone, respectively.

Previous work has shown that thalamic neurones (0.7 mg kg\(^{-1}\)) have a higher sensitivity than the medullary dorsal horn (2.5 mg kg\(^{-1}\)) to systemic morphine, suggesting that the effects of morphine on thalamic neurones do not arise as a consequence of a spinal site of action (Hill et al., 1982). Nevertheless it is feasible that the effects of systemic morphine are produced through sites of action at various levels of the pain pathway. Indeed intraplantar and intravenous injection of morphine produced similar anti-nociceptive effects in the carrageenan model of inflammation.
(Perrot et al., 2001) and a high dose of systemic morphine \( (3 \text{ mg kg}^{-1}) \) attenuated carrageenan-evoked expression of c-Fos at the level of the spinal cord (Catheline et al., 1999). Thus it is important to ascertain the effect of direct intra-thalamic administration of morphine.

In our study, intra-thalamic morphine produced inhibitory effects on noxious-evoked responses of VPL neurones in naïve rats and rats with hindpaw inflammation that were similar to those observed with systemic morphine, suggesting that the effects of systemic morphine may be mediated by sites of action including the VPL. In contrast to systemic morphine, intra-thalamic morphine did not produce marked inhibitions of low weight-evoked responses of VPL neurones in rats with hindpaw inflammation, suggesting that the effect of systemic morphine on this response is mediated by other sites of action. This effect may reflect peripheral sensitization resulting in these normally innocuous stimuli activating pain pathways, which are sensitive to opioids. Indeed, systemic morphine has an increased potency on threshold responses in the carrageenan model of inflammatory pain (Joris et al., 1990; Ossipov et al., 1995), which is likely to be mediated by peripheral (Kayser et al., 1991) and spinal sites of action (Stanfa and Dickenson, 1993).

A major finding of our study of the effects of morphine in rats with hindpaw inflammation was that higher weight noxious-evoked responses of VPL neurones had a reduced sensitivity to systemic and intra-thalamic morphine, compared to naïve rats. In parallel with this reduced opioid sensitivity, we observed significantly larger responses of VPL neurones to noxious (60 and 80 g) mechanical stimuli in rats with hindpaw inflammation. As described earlier, there is evidence for a role of thalamic glutamate and NMDA receptors contributing to inflammatory hyperalgesia and previous studies have suggested that increased activation of NMDA receptors may contribute to a reduced sensitivity to morphine (Chapman et al., 1994; Nichols et al., 1997). Thus, it is feasible that due to the increased magnitude of evoked responses, which is likely to involve activation of NMDA receptor, a higher dose of morphine is required to inhibit these responses of VPL neurones.

MAP was also measured in this study. The lowest dose of morphine used did not alter MAP at any time point. Higher doses of morphine (1 and 2 mg kg\(^{-1}\)) decreased MAP at 1 min post-administration, but not at 10 and 20 min post-administration, time-points which correspond to maximal effects of morphine on mechanically-evoked responses of neurones. Thus, it is unlikely that cardiovascular effects of morphine account for the effects of morphine on responses of VPL and Po neurones.

In conclusion, we report significant changes in mechanically-evoked responses of VPL neurones, but not Po neurones, in naïve rats and rats with hindpaw inflammation. The reported facilitated noxious-evoked responses of VPL neurones are associated with a reduced sensitivity to morphine and may contribute to hyperalgesia associated with hindpaw inflammation.

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**References**


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