Effects of local anaesthetics on carrageenan-evoked inflammatory nociceptive processing in the rat

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Summary
We have assessed the effects of intraplantar local anaesthetics (bupivacaine and lignocaine) on carrageenan-induced oedema, mechanical allodynia and spinal c-fos protein expression. Mechanical allodynia was evaluated using the vocalization threshold to paw pressure (VTPP) every 30 min until 60, 180 or 240 min after administration of carrageenan. Peripheral oedema, mechanical allodynia and spinal c-fos protein expression were maximal 180 min after carrageenan. Lignocaine did not influence either oedema or VTPP, but reduced spinal c-fos expression at 60 min after carrageenan without later effects. Bupivacaine induced an increase in VTPP at 30 and 60 min, limitation of oedema at 60 and 180 min, but these effects were not present 240 min after carrageenan. Intraplantar infiltration with lignocaine and bupivacaine before carrageenan transiently limited signs of inflammatory pain but did not prevent them. (Br. J. Anaesth. 1996; 77: 645–652)

Key words

Bupivacaine and lignocaine, long- and short-acting local anaesthetics respectively, are used in both acute and chronic pain therapy (see references in Bonica and Butler). There is evidence that pain processing may be reduced by pre-administration of various agents (e.g. opioids, local anaesthetics, non-steroidal anti-inflammatory drugs (NSAID)) leading to the concept of pre-emptive analgesia. However, this concept is still debated and few clinical studies have confirmed the clinical significance of pre-emptive analgesia. Administration of local anaesthetics before tissue injury may prevent peripheral and central sensitization of the nervous system induced by nociceptive input (see references in Codere and colleagues). The pre-emptive analgesic effect of local anaesthetics has been observed in different animal models of pain (see references in Codere and colleagues).

In this study, we have investigated the possible pre-emptive effects of local anaesthetics (lignocaine and bupivacaine) in the carrageenan model of inflammatory pain in rats. As shown previously, intraplantar injection of carrageenan induces ipsilateral inflammation (see references in Kocher and colleagues) associated with mechanical allodynia and c-fos-protein-like immunoreactivity (c-fos LI) in the ipsilateral dorsal horn of the spinal cord. The method based on the immunoreactivity of c-fos-protein is used widely as a form of neuronal-activity mapping (see references in Morgan and Curran). For a recent review of the mechanisms of expression of c-fos protein within the nervous system see Hughes and Draganow. Many studies following the original report have provided evidence that various noxious stimulations evoked expression of c-fos protein in spinal neurones (see references in Zimmermann and Herdegen), thus providing an indirect marker of neurones involved in spinal nociceptive transmission. In our previous studies, we used immunohistochemical revelation of spinal c-fos expression in the carrageenan model of inflammatory pain to assess the effects of analgesic/anti-inflammatory drugs, including systemic and intraplantar morphine and various systemic NSAID (see Buritova and colleagues and references therein). In this study, we have combined behavioural and immunohistochemical approaches to investigate the effects of pre-administered intraplantar local anaesthetics in carrageenan-induced inflammatory pain in rats.

Materials and methods
EXPERIMENTAL ANIMALS
Experiments were performed on 52 adult male albino Sprague–Dawley rats (Charles River, France; 48 carrageenan-stimulated and four non-stimulated rats), weighing 225–250 g. Rats were acclimatized to the laboratory for 8 days before the experiment. Rats were tested using the vocalization threshold to paw pressure (VTPP) and were trained in the experimental conditions during the last 2 days before the experiment.
ethics guidelines of the IASP were followed for investigations of experimental pain in conscious animals.20

DRUG ADMINISTRATION
Peripheral inflammation was induced by intraplantar injection of carrageenan (λ-carrageenan, Sigma; 6 mg per 150 μl of saline (0.9% NaCl)) in the right hind paw of the non-anaesthetized rat. In this study, control rats receiving intraplantar injection of saline were not included as we have shown previously negligible spinal c-fos expression after intraplantar saline (<5 c-fos LI neurones per section L4–L5) which was not significantly different from that observed in non-stimulated rats.12 The preliminary unpublished study demonstrated that intraplantar administration of local anaesthetics (0.5% Marcaine, 2% xylocaine) without carrageenan injection did not induce spinal c-fos expression significantly different from that observed in intraplantar saline injected rat (<5 c-fos LI neurones per section L4–L5).

Intraplantar bupivacaine (0.5% Marcaine, Astra, France) and lignocaine (2% Xylocaine, Astra, France) were injected into the right hind paw of non-anaesthetized rats (200 μl for both drugs) 5 min before intraplantar injection of carrageenan. The timing, concentration and volume of local anaesthetic were chosen according to a previous study.22 as it represents a more integrated nociceptive behaviour than paw withdrawal. The choice of a cut-off value was necessary to limit injury to the paw and excessive stimulation of the nociceptors. The cut-off value was 600 g and was considered sufficient to represent an anaesthetic state.

IMMUNOHISTOCHEMISTRY FOR EVALUATION OF SPINAL C-FOS PROTEIN EXPRESSION
As demonstrated previously, carrageenan-evoked spinal c-fos expression was detected at 60 min and was maximal at 180 min after intraplantar injection of carrageenan. Therefore, in this study of the time course of the local anaesthetic effects, rats were perfused at various times after injection of carrageenan: 60, 180 and 240 min, immediately after the last test of VTPP. As described previously (see references in Buritova and colleagues19), rats were deeply anaesthetized (pentobarbitone (Sanofi) 55 mg kg−1 i.p.) and perfused intracardially with phosphate-buffered saline (PBS) 0.1 mol litre−1 followed by 4% paraformaldehyde in phosphate buffer (PB) 0.1 mol litre−1. The spinal cord was removed, post-fixed for 4 h and cryoprotected in 30% sucrose overnight. Frozen serial frontal sections (40 μm) of the lumbar L4–L5 segments were cut. Immunohistochemistry of the free floating sections was performed with polyclonal antiserum, generated in rabbits, directed against the c-fos protein (Oncogene Science Inc., Ab-2 solution 0.1 mg ml−1 diluted 1:4000), using the conventional avidin–biotin–peroxidase complex method with visualization by 1-naphthol ammonium carbonate solution (for more details see methods in Buritova and colleagues15).

COUNTING OF SPINAL C-FOS LI NEURONES
As described previously (see Buritova and colleagues19 and references therein), distribution of c-fos LI neurones was studied in four defined regions: superficial laminae (laminae I–II), nucleus proprius (laminae III–IV) and neck (laminae V–VI) of the dorsal horn and, in addition, ventral horn (laminae VII–X; ventral) of the spinal cord. For each rat, two counts were made: (1) the total number of c-fos LI neurones in the grey matter for 10 sections through L4–L5 segments, and (2) in these 10 sections, the number of c-fos LI neurones in the four defined regions. Plotting and counting the c-fos LI neurones were performed blind to the experimental condition. Statistical analysis was performed using analysis of variance (ANOVA) and the Fisher’s protected least squares difference test (Fisher’s PLSD test) for multiple comparisons. The effects of local anaesthetics on peripheral oedema, VTPP and number of spinal c-fos LI neurones were determined compared with controls (control group).

EXPERIMENTAL DESIGN
To evaluate the time course of the effects of local anaesthetics on carrageenan-induced inflammatory nociceptive processing, the experiment was per-
formed in three different series. In each series, rats were perfused immediately after the last test of VTPP.

In the first series (n = 18), rats in the control, lignocaine and bupivacaine groups were tested for VTPP every 30 min for 60 min after administration of carrageenan at which time rats were perfused. In the second series (n = 18), rats in the control, lignocaine and bupivacaine groups were tested for VTPP every 30 min for 180 min after administration of carrageenan at which time rats were perfused. In the third series (n = 12), control and bupivacaine groups were tested for VTPP every 30 min for 240 min after administration of carrageenan at which time rats were perfused. In this series, the lignocaine group was not included as intraplantar pre-administration of lignocaine was not efficacious as early as 180 min after carrageenan administration.

In these experimental series, three distinct groups of rats were defined: in the control group (after carrageenan administration of lignocaine was not efficacious as early as 180 min after carrageenan), the lignocaine group (0.02) cm was reduced slightly compared with the control group, carrageenan-evoked ankle oedema (0.75 (0.1) cm, respectively) and ankle (0.8 (0.1), 1.2 (0.1) and 1.1 (0.1) cm, respectively) diameters were increased significantly compared with carrageenan non-stimulated rats (mean values for paw and ankle diameters: 0.5 (0.1) and 0.7 (0.1) cm, respectively). Inflammatory oedema was not present in the contralateral hind paw at each time.

At 60 min after carrageenan, in the bupivacaine group, carrageenan-evoked ankle oedema (0.75 (0.02) cm) was reduced slightly compared with the control group (P < 0.01) but paw oedema was not influenced. Bupivacaine had no effect on paw or ankle oedema at 180 and 240 min after carrageenan.

Results

DEVELOPMENT OF CARRAGEENAN-EVOKED OEDEMA

In the control group, 60, 180 and 240 min after intraplantar administration of carrageenan, both paw (0.8 SEM 0.1), 1.1 (0.1) and 1.1 (0.1) cm, respectively) and ankle (0.8 (0.1), 1.2 (0.1) and 1.1 (0.1) cm, respectively) diameters were increased significantly compared with carrageenan non-stimulated rats (mean values for paw and ankle diameters: 0.5 (0.1) and 0.7 (0.1) cm, respectively). Inflammatory oedema was not present in the contralateral hind paw at each time.

In the control group, 60, 180 and 240 min after intraplantar carrageenan. Significance compared with control group (P = 0.001). The study of the effect of lignocaine at 4 h was not performed.

CARRAGEENAN-EVOKED DECREASE OF VTPP

The results were similar for the three series and were therefore pooled (see fig. 1).

Intraplantar carrageenan decreased VTPP at each time, with a final decrease to 35% of control values at 240 min after carrageenan (fig. 1). Overall, VTPP decreased from 288 (7) g at time 0 min, to 100 (3) g at 240 min after carrageenan (fig. 1).

In the lignocaine group, VTPP never reached the cut-off value at each time after carrageenan. At 60 and 180 min after carrageenan, VTPP decreased to 76% and 37% of control values, respectively, reflecting the development of mechanical allodynia. Overall, VTPP decreased from 287 (6) g at time 0 min to 108 (9) g at 180 min after carrageenan (fig. 1).

In the bupivacaine group, VTPP reached the cut-off value in 95% of rats at 60 min after carrageenan. At later times, 180 and 240 min, VTPP decreased to 37% and 35% of control values, respectively. Overall, VTPP decreased from 287 (6) g at time

![Figure 1](image.png)

**Table 1** Effects of intraplantar 0.5% bupivacaine and 2% lignocaine on the number of c-fos LI neurones in the superficial (I–II) and deep (V–VI) laminae of the dorsal horn and in the ventral horn (Ventral) of the L4–L5 segments of the rat spinal cord at 1, 3 and 4 h after intraplantar carrageenan. Results are expressed as the absolute mean (SEM) of the number of c-fos LI neurones per section for each region. Significance of the effects of both anaesthetics, compared with the control group, was performed using ANOVA and Fisher’s PLSD test (**P < 0.01, ***P < 0.001). The effect of lignocaine at 4 h was not performed.

<table>
<thead>
<tr>
<th>No. c-fos/LI neurones/section of L4–L5 segments</th>
<th>1 h after carrageenan</th>
<th>3 h after carrageenan</th>
<th>4 h after carrageenan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Total (n)</td>
<td>I–II</td>
<td>V–VI</td>
</tr>
<tr>
<td>Control</td>
<td>101 (8)</td>
<td>45 (3)</td>
<td>38 (5)</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>40 (3)**</td>
<td>16 (3)**</td>
<td>16 (1)**</td>
</tr>
<tr>
<td>Lignocaine</td>
<td>64 (6)**</td>
<td>25 (3)**</td>
<td>23 (2)**</td>
</tr>
</tbody>
</table>
0 min to 100 (3) g at 240 min after carrageenan (fig. 1). The mean duration of analgesic effect of bupivacaine was 55 (16) min.

TIME COURSE OF CARRAGEENAN-EVOKED SPINAL C-FOS EXPRESSION

In the control group, the total number of c-fos LI neurones was greatly increased in the ipsilateral spinal cord at 60, 180 and 240 min after carrageenan (fig. 2, fig. 4A; table 1). At all times after carrageenan, spinal c-fos LI neurones of the control group were located predominantly in laminae I–II and V–VI of the dorsal horn of the lumbar spinal cord (fig. 2, fig. 4A, C, table 1). At 60, 180 and 240 min after carrageenan, the number of c-fos LI neurones in the nucleus proprius (laminae III–IV: 6 (1), 10 (2) and 8 (1) c-fos LI neurones per L4–L5 section, respectively) was moderate compared with the total number of c-fos LI neurones per L4–L5 section (101 (8), 200 (15) and 141 (6), respectively) (table 1). At each time, c-fos LI neurones were almost absent in the contralateral spinal cord (< 5 c-fos LI neurones per L4–L5 section).

At 60 min after intraplantar carrageenan, carrageenan-evoked spinal c-fos expression in the bupivacaine and lignocaine groups was significantly different from that observed in the control group, for both the total number of c-fos LI neurones in segments L4–L5 (F (3, 20) = 16.17; P < 0.001) and their laminar distribution (F (3, 80) = 34.20; P < 0.001). Intraplantar pre-administration of both bupivacaine and lignocaine reduced the total number of c-fos LI neurones induced at 60 min after carrageenan (60 (3) % and 37 (6) % reduction of control group values, respectively; P < 0.001 for both drugs) (fig. 4A, table 1). Furthermore, in both the bupivacaine and lignocaine groups, the significant reduction in the number of superficial (laminae I–II: 63 (6) % and 44 (6) % reduction of control group values, respectively; P < 0.001 for both drugs) (fig. 3) and deep (laminae V–VI: 59 (3) % and 39 (5) % reduction of control group values, respectively; P < 0.001 for both drugs) c-fos LI neurones was observed.
at 60 min after carrageenan (fig. 4A-C, table 1). Therefore, intraplantar pre-administration of both bupivacaine and lignocaine had a similar effect on the number of c-fos LI neurones at 60 min after intraplantar carrageenan (fig. 4A-C, table 1). In contrast, in the lignocaine group, the number of spinal c-fos LI neurones induced at 180 min after carrageenan was not affected (table 1).

Intraplantar pre-administration of bupivacaine did not influence spinal c-fos expression induced at 240 min after carrageenan (table 1). As intraplantar lignocaine did not influence spinal c-fos expression at 180 min after carrageenan, the study
of its effect at 240 min after carrageenan was not performed.

**Discussion**

Intraplantar administration of a long-acting local anaesthetic, bupivacaine, postponed mechanical allodynia and spinal c-fos protein expression associated with the inflammatory state induced by intraplantar injection of carrageenan in rats, while a short-acting local anaesthetic, lignocaine, reduced only transiently carrageenan-induced spinal c-fos protein expression. Overall, the effects of intraplantar bupivacaine and lignocaine in the carrageenan model of inflammatory pain were related to the duration of action of the anaesthetics.

**TIME COURSE OF VOCALIZATION THRESHOLD AND OEDEMA**

In accordance with previous studies (Kayser and Guilbaud, see references in Buritova and colleagues) intraplantar injection of carrageenan induced ipsilateral oedema, without influencing the contralateral hind paw, at all times. The lack of effect of pre-administered intraplantar 2% lignocaine 200 μl on either oedema or VTPP after carrageenan was in concordance with previous studies showing the short duration of the antinociceptive effect of similar doses of lignocaine in behavioural experiments using the carrageenan model of inflammatory pain (18 min after 2% lignocaine 50 μl) or formalin response in an orofacial pain model (6 min after 2% lignocaine 50 μl).

In a carrageenan model of inflammatory pain, analgesia was defined previously as VTPP within the range of usual variability of the control value in normal situations (i.e. 85–115% of control value). In this study, the duration of analgesic action of 0.5% bupivacaine 200 μl was estimated to be 55 (16) min after carrageenan. Similarly, pre-administered intraplantar bupivacaine reduced ankle oedema evoked at 60 min after carrageenan but this effect did not persist. The combined effect of bupivacaine on extension of oedema and reduction of VTPP may be related, at least in part, to the transient abolition of the axon reflex involved in the development and extension of inflammation. The mechanism of action of bupivacaine involves binding of bupivacaine to voltage-dependent Na+ channels which reduces neuronal transmission, axon reflex and consequently development of inflammation (see Butterworth and Strichartz and references therein). In addition, local anaesthetics have been shown to block peripheral release of inflammatory substances such as substance P. Thus the mechanisms mentioned above may be involved in the transient peripheral anti-inflammatory effect of intraplantar bupivacaine. The limited extent of peripheral oedema in the bupivacaine group, at 60 min after carrageenan, corresponded to the peripheral analgesic effect of bupivacaine during the period of its action. Overall, the peripheral effects of local anaesthetics probably reduced the nociceptive inputs and consequently influenced carrageenan-induced spinal c-fos expression.

**TIME COURSE OF SPINAL C-FOS EXPRESSION**

In the control group, spinal c-fos expression at 60, 180 and 240 min after intraplantar carrageenan was in agreement with our previous study showing the time course of spinal c-fos expression in the same model of inflammatory pain (see references in Buritova and colleagues). This carrageenan evoked spinal c-fos expression was localized predominantly in the superficial (I–II) and deep (V–VI) laminae of the dorsal horn of the spinal cord in accordance with the spinal areas containing neurones activated by noxious stimuli driven by C- and Aδ-fibres which evoked the spinal c-fos expression.

Pre-administration of lignocaine attenuated spinal c-fos expression in both superficial and deep laminae at 60 min after carrageenan. This effect did not persist and the maximal number of c-fos neurones similar to the control group was reached at 180 min after carrageenan. This effect is in keeping with an electrophysiological study showing that administration of 2% lignocaine 50 μl s.c. into the site of peripheral injection of formalin completely blocked the first phase of the response to formalin of the deep dorsal horn neurones (about the first 10 min) without affecting second phase activity. Thus 2% lignocaine 50–200 μl seems to be sufficient to prevent transmission of nociceptive inputs during the initial phase of nociceptive primary afferent activation with consequent attenuation of the time pattern of spinal c-fos expression. In this study, the reduced number of c-fos LI neurones at 60 min may reflect both a short duration of block of noxious input produced by lignocaine at the initial phase of inflammation and c-fos protein synthesis delay related to delay of c-fos messenger RNA (mRNA) expression about 30 min after carrageenan.

In the bupivacaine group, the reduction in spinal c-fos expression in both superficial and deep laminae was observed 60 min after carrageenan. At 180 min after carrageenan when the reduction in VTPP, reflecting mechanical allodynia, was equivalent to control group values, c-fos expression in the superficial laminae was similar to the control group. However, at this time the total number of c-fos LI neurones and c-fos expression in deep laminae were lower than in the control group. This laminar pattern is not surprising as c-fos protein is initially expressed in superficial neurones, and subsequently in the neurones in the deep laminae of the spinal dorsal horn at a later phase of noxious stimulus transmission, as has been shown after intraplantar injection of formalin and carrageenan. Thus, in this study, the laminar pattern of carrageenan-induced spinal c-fos expression, influenced by intraplantar administration of both bupivacaine and lignocaine, may result from peripheral block of nociceptive input by local anaesthetics and consequently delayed c-fos expression at the spinal cord level.

Interestingly, the effect of pre-administered intraplantar bupivacaine on the laminar pattern of carrageenan-induced spinal c-fos expression at 60 min after carrageenan was similar to that observed with pre-administered intraplantar morphine and
various systemic NSAID (see references in Buritova and colleagues) observed previously in the same experimental paradigm at 180 min after carrageenan. In these previous studies, the period of 180 min after intraplantar carrageenan had been covered by activity of the analgesic/anti-inflammatory drugs which resulted in reduction of c-fos expression in both superficial and deep laminae of the spinal dorsal horn. Overall, during the period of drug activity, both bupivacaine (60 min after carrageenan) and analgesic/anti-inflammatory drugs (180 min after carrageenan (see references in Buritova and colleagues) had a similar effect on reducing spinal c-fos expression in both superficial and deep laminae. In contrast, after the period of drug activity (e.g. at 180 min after carrageenan), administration of bupivacaine induced a dissociative laminar pattern of spinal c-fos expression without effect on c-fos expression in superficial laminae. This dissociative effect of bupivacaine was similar to that described previously with the NMDA receptor antagonist, (−)-HA966, in the same experimental paradigm. Interestingly, bupivacaine and (−)-HA 966 have a similar duration of antinociceptive effect (about 60 min).

In this study, the absence of persistent effects of intraplantar local anaesthetic on both behavioural nociceptive test and spinal c-fos expression in the superficial laminae does not support the hypothesis of efficacy of pre-emptive analgesia. However, the reduction in both total and deep laminae c-fos expression by bupivacaine may have an influence on the development of chronic pain states but additional studies are required to test this hypothesis.

LOCAL ANAESTHETICS AND PREVENTION OF PAIN

In this study we used parallel behavioural and immunohistochemical approaches to assess the possible pre-emptive effect of local anaesthetics. The reduction in spinal c-fos expression in both the superficial and deep laminae at 60 min suggests that local anaesthetic pre-infiltration interrupted nociceptive inputs during the initial phase of carrageenan inflammation. However, a longer block may be necessary to reveal a pre-emptive analgesic effect of local anaesthetics. Thus an increase in the dose of local anaesthetic used for infiltration or a nerve block technique may be proposed. Unfortunately, re-injection in this study would have been toxic (1 mg of bupivacaine was already injected; i.e. more than 4 mg kg−1). Furthermore, we have previously used 0.5% bupivacaine with adrenaline or higher doses (5 mg of bupivacaine instead of 1 mg in 200 μl) of bupivacaine included in microspheres which produced, respectively, a 100% and 175% (unpublished data) increase in analgesia related to infiltration but without a pre-emptive effect detected by behavioural tests in the same carrageenan model. In contrast, the intervention required for nerve block may induce additional nociceptive stimulation which influences carrageenan-induced spinal c-fos expression and consequently impairs the homogeneity of control values. In addition, the behavioural test used in this study (vocalization threshold to paw pressure) may be difficult to evaluate when there is total paralysis of the limb.

The development of inflammatory nociception induced by intraplantar carrageenan in the rat is comparable to the time course of postoperative pain (24–96 h) and it might explain the similar results of this study and clinical studies on pre-emptive analgesia with local anaesthetic infiltration of the site of superficial surgery (see references in Dahl and Kehlet). Our results extend clinical studies showing that pre-emptive anaesthesia with infiltration by local anaesthetics has no long-term benefits for postoperative analgesia (see references in Dahl and Kehlet).

In summary, the effects of intraplantar pre-administration of bupivacaine and lignocaine on carrageenan-induced mechanical allodynia did not persist beyond the direct local anaesthetic effect. Similarly, carrageenan-induced nociceptive inputs were reduced only briefly by local anaesthetics, as shown by the transient reduction in spinal c-fos expression, an indicator of nociceptive transmission at the spinal cord.

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