Radiotelemetric and Symptomatic Evaluation of Pain in the Rat After Laparotomy: Long-Term Benefits of Perioperative Ropivacaine Care

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Abstract: Effective relief of acute and long-term postoperative pain is of utmost importance to patients undergoing surgery. Here, we worked on a controlled procedure of abdominal surgery in the rat inducing persistent postoperative pain symptoms for up to 10 days and tested the efficacy of perioperative care with the local anesthetic ropivacaine. Laparotomy was likewise used to implant radiotelemetric probes by which electrocardiogram, body temperature, and locomotor activity were recorded in freely moving animals. We showed that postoperative pain symptoms (mechanical allodynia) measured in periphery of the scar were associated over time with persistent tachycardia, elevated heart rate variability, and loss of mobility. Furthermore, a single subcutaneous infiltration of the local anesthetic ropivacaine in the periphery of the abdominal incision was sufficient to prevent the appearance of allodynia and the associated cardiac and motor signs of pain, monitored by radiotelemetry. These beneficial effects were observed when the infiltration was performed in the perioperative period, but not later. This study on freely moving animals exhibiting long-lasting postoperative pain symptoms and altered autonomic/motor function illustrates well the importance of the timing of preemptive analgesia care with long-acting local anesthetics. Moreover, it emphasizes the utility of monitoring heart rate variability to quantify spontaneous expression of long-lasting postoperative pain.

Perspective: Speeding the recovery time after surgery using perioperative ropivacaine care is of significant clinical relevance because it might limit the risk of chronic pain and postoperative complications. In humans, chronobiological analysis of heart rate variability could also help quantify spontaneous pain expression with minimal emotional bias.

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simultaneously in pertinent animal models of postoperative pain. There are none which directly describe the time-course correlation between pain-related changes in physiological parameters, behaviors, and sensory-motor threshold sensitivity (allodynia and/or hyperalgesia) around the scar. Apart from monitoring pain-associated alteration of locomotion, skin conductance, or body temperature, pain expression has been successfully related to excessive heart rate variability in anesthetized humans and infants.

It is, therefore, particularly important to focus on cardiovascular parameters while using animal models of acute and persistent pain. In most pain models, no changes in the mean heart rate can be detected and, based on human studies, the heart rate variability (eg, variability between RR waves of the ECG) is postulated to be an interesting alternative pain marker. Until now, there are little data available regarding its possible use to monitor postoperative recovery from surgery in relation with the progressive disappearance of pain symptoms and the efficacy of perioperative analgesia care.

There is a vast and controversial literature regarding the clinical efficacy of perioperative analgesia care aimed at improving recovery from surgery. At the same time, perioperative analgesia is strongly recommended and frequently, if not systematically, prescribed at the hospital. The reason is that it is theoretically expected that a reduction in the excitability of freshly-cut peripheral sensory nerves will prevent an excessive peripheral/central sensitization and, therefore, will presumably reduce pain expression as well as recovery time from surgery. In contrast to many animal studies, data from human trials do not fully support this hypothesis and point out the importance of timing for the analgesia care. Among the numerous drugs that are used during the perioperative period in humans, local anaesthetics are efficient to block the peripheral afferent neuronal barrage of nociceptive messages resulting from surgery. In many instances, they have been used with success to prevent episodes of pain seen immediately after awakening (ie, 24 hours after surgery) and more rarely at later time points.

In animal models, beneficial effects of perioperative analgesia care were found following injections of local anaesthetics in studies of the tonic inflammatory nociceptive responses to intraplantar formalin, of the autotomy frequency rates following sciatic nerve transaction, or of the failed back-surgery syndrome. The first objective of the present study was to identify and quantify spontaneous pain expression after major abdominal surgery using a radiotelemetric monitoring of several physiological parameters in freely-moving animals. These physiological changes were further considered as being spontaneous signs of pain if they were correlated over time with the presence of a skin hyperalgesia around the scar and sensitive to the alkaloid morphine, the nonsteroidal anti-inflammatory drug ketoprofen, and the local anesthetic ropivacaine. In a second step, we also sought to test the efficacy of a perioperative pain care using the local anesthetic ropivacaine, a compound that has not been characterized with a combined symptomatic and physiological approach in a major surgery involving skin, muscles, and peritoneum incisions. We chose ropivacaine because it exerts a preferred action on tetrodotoxin-resistant voltage-gated sodium channel subtypes that are expressed by small unmyelinated C-fibres (and neurons) in physiological conditions and are submitted to major plastic changes in pathological pain situations. Moreover, ropivacaine displays limited motor block, has no effect on cardiovascular parameters, and, when used acutely, shows little central nervous system and cardiac toxicity.

Methods

Ethical Approval

All experiments were conducted in conformity with the recommendations of the European Committee Council Direction of November 24, 1986 (86/609/EEC), with an authorization for animal experimentation from the French Department of Agriculture (License 67-116 to P.P.) and with the agreement of the regional ethical committee (authorization number AL/10/13/03/07).

Animals

Male Sprague-Dawley rats (Janvier, Le Genest-Saint-Isle, France) weighing ~350 g at the beginning of experiments were used for this study. Animals were housed individually under standard conditions (room temperature 22°C; 12 hour light/dark cycle; lights on at 0700) with ad libitum access to food and water. Before the onset of the experiment, all animals were subject to at least 1 week habituation to the experimental room, to handling, and to behavioral testing. Behavioral tests were done during the light period (between 1000 and 1600 hours).

Surgical Implantation of the Radiotelemetric Probe

Implantation of the radiotelemetric transmitter (model TA11CTA-F40; Data Sciences International, St Paul, MN) was performed under sterile conditions while animals were deeply anesthetized with a cocktail of ketamine (87 mg/kg; Imalgène 1000; Merial, Lyon, France) and xylazine (13 mg/kg; Sigma, St Louis, MO). The surgery field was shaved and carefully cleaned with povindone iodine dermal solution (Betadine Scrub 4%; Viatris, Mérignac, France). A first incision of about
2 cm was made medially through the skin, the abdominal muscle and the peritoneum to implant the transmitter. Inside the peritoneal space, the transmitter was tightly stitched to the abdominal muscles with 3 points using a nonresorbing suture (Silcam 4/0, Braun, France). The 2 electrodes used to record the electrocardiogram (ECG) were placed under the skin and firmly sutured to the muscles at the level of the last left rib and under the right axilla. Two cutaneous incisions allowing placement of the ECG electrodes were sutured and cleaned with betadine. A precise 50-minute surgery time was respected, and incised muscles were retracted during this whole period. Transmitters were activated immediately after surgery in order to monitor mobility, heart rate, and abdominal temperature from the freely-moving animals during the postoperative recovery period.

**Radiotelemetry Analysis**

Mobility, heart rate, and abdominal temperature were recorded using DSI Data Exchange Matrices (Data Sciences International). Data were transmitted on-line using radio frequency waves at 455 kHz and stored on a remote-controlled computer. Animal locomotor activity (in arbitrary unit, AU), heart rate (beats per minute, bpm) and abdominal temperature (°C) were calculated every minute during a recording waveform of 20 seconds at an acquisition rate of 1 kHz for each animal, using DataQuest ART 4.0 (Data Sciences International). After acquisition, data were exported as Excel and text files for further analysis.

Noninvasive baseline measurements of mobility were also made with the Activ-Meter (Bioseb, Chaville, France) actimetry measurement system. Stable mobility values for telemetry-implanted rats, 15 to 20 days after surgery, were similar to the baseline values for naive rats (data not shown).

Rhythograms were constructed by double-plotting successive 24-hour periods (horizontal scale) over days after surgery (vertical scale). Mobility was displayed on standard actograms with scale cutoff at 20 AU. Pseudo-color scales were used for heart rate and temperature. Mean graphs were built by calculating the average of each parameter over the 12 hours of each day (7–19 hours, light period) or night (19–7 hours, dark period) period.

For heart rate variability (HRV) analysis, RR intervals and heart rate were calculated for successive segments of 20-second electrocardiogram recording (ie, giving more than 100 PQRST complexes given the heart rate of rats), over a period of 20 minutes. R waves were automatically detected and individual errors were manually checked using Clampfit 10.2 software (Molecular Devices, MDS Analytical Technologies, Toronto, Canada) before extracting RR intervals. Time domain analysis of heart rate variability (HRV) allowed to obtain SD1, 1 descriptor of the Poincare plot for RR intervals, describing the short-term HRV. SD1 was calculated as SD1 = SDSD/√2, where SDSD is the standard deviation of the successive differences of the RR intervals, \( \Delta RR_n \) = RRn–RRn–1  10% of the mean value. They are referred in the text as pNN18, expressed in % and correspond to an arbitrary threshold change in the RR interval of 18 ms.

**Evaluation of Postoperative Pain Symptoms**

Before the beginning of experiment, animals were habituated to handling and testing for about 1 week. Touch test von Frey filaments with logarithmic incremental stiffness (4, 6, 8, 10, 15, 26, 60 g; Bioseb, Chaville, France) were used to determine the noceptive mechanical threshold at the periphery of the abdominal scar. Animals were placed in clear Plexiglas (Arkema Inc, Philadelphia, PA) enclosures (24 × 14 × 30 cm) on an elevated metallic mesh. After 15 minutes habituation to the testing box, filaments with increasing stiffness were successively applied at a time interval of at least 30 seconds and after full return of the animal to its resting behavior. Each filament was applied 5 times during 2 seconds with a test-free interval of 5 seconds at the periphery of the abdominal scar. The noceptive pressure threshold was reached when the animal exhibited a minimum of 3 aversive behavioral responses for 5 consecutive stimuli (based on Chapman et al). Licking of the scar, abdominal withdrawal, or escape behavior were all counted as aversive responses. The 26-g filament was the stiffest tested (ie, maximum measured value), because the 60-g filament was too stiff to bend when applied on rat abdomen. The von Frey tests were conducted blind with respect to the treatments received by animals.

**Drugs**

Postoperative pain symptoms sensitivity to some analgesics was characterized after a unique intradermal injection (300 \( \mu l \)) around the scar of ketoprofen (Centravet, Taden, France; 10 mg/kg), morphine hydrochloride (Sigma Aldrich, St Louis, MO; 5 mg/kg) or ropivacaine (Naropeine; AstraZeneca, France; 2 mg/mL). Mechanical von Frey thresholds were measured 1 hour after the injection. Perioperative ropivacaine care was evaluated with single systemic administration or subcutaneous injection around the scar (300 \( \mu l \); 2 mg/mL), before awakening of the animal, before, or after surgery. For all injections done in hyperalgesic animals, we used short-term and rapidly reversible anesthesia with 3% halothane (Belamont, Paris, France) to limit stress. Animals were tested for mechanical nociception 30 minutes later.

**Statistical Analysis**

Data in text are expressed as mean ± standard deviation. Mean values of the mobility, heart rate, temperature or mechanical hypersensitivity were compared at different times and between treatment groups. Repeated-measures 2-way or 3-way ANOVA, with factors treatment (between), time and day/night (within), were obtained for all segments provided a global SD1 for each animal, as well as an estimation of its variability, SD(SD1). Part of this variability was found to arise from occasional large alterations of the RR interval which were clearly not artifactual. We therefore counted, for each 20-second segment, the number of \( \Delta RR_n \) values larger than ∼10% of the mean value. They are referred in the text as pNN18, expressed in % and correspond to an arbitrary threshold change in the RR interval of 18 ms.
When the ANOVA test was significant, the Tukey test was used for post hoc multiple comparisons between individual groups. The ordinal data from von Frey experiments were analyzed with nonparametric statistics on ranks using KyPlot 2.15 (KyensLab, Tokyo, Japan). Friedman test for the effect of time within a given treatment group and Wilcoxon-Mann-Whitney or Kruskal-Wallis test for between-groups comparison at a given time were used, followed by Steel test for multiple comparisons. Differences were considered significant for $P < .05$.

Results

**Time-Course of Postoperative Abdominal Hyperalgesia and Sensitivity to Analgesics**

The long-lasting procedure of laparotomy used in this study to implant radiotelemetric probes is obviously expected to induce a significant abdominal incisional pain. In a first set of experiments, we measured the evolution of mechanical nociceptive thresholds with von Frey's filaments applied around the scar (see Methods) during the postoperative period (Fig 1A). At day 1 after surgery, the mean pressure threshold to induce a nociceptive response was $8.0 \pm 1.6$ g ($n = 4$), a value corresponding to severe hyperalgesia when compared to values found in the same animals after shaving before surgery ($20.5 \pm 6.4$ g, $n = 4$). Rats remained hyperalgesic for about 9 days and normal mechanical threshold values of the skin to nociceptive pressure stimulus were recovered after 10 days (Fig 1A).

We next tested the sensitivity of postoperative pain symptoms to various analgesics (Fig 1B). This was done at postoperative day 3, where von Frey threshold mean value was $8.3 \pm 1.3$ g ($n = 8$), indicating that the animals were still hyperalgesic. One hour after a single intradermal infiltration around the scar of the long-lasting local anesthetic ropivacaine (2 mg/kg), mean von Frey threshold reached its maximum cut-off value of $26.0$ g ($n = 8$). This full antihyperalgesic effect of ropivacaine, however, was transient, returning back to a hyperalgesic value of $10.0 \pm 3.3$ g after 24 hours (Fig 1B; day 4; $n = 8$). Similarly to ropivacaine action, the intradermal infiltration of morphine around the scar (5 mg/kg) induced a strong anti-hyperalgesic effect: The von Frey threshold reached the maximum cut-off value of $26.0$ g ($n = 8$) 30 minutes after infiltration, whereas this threshold was $7.8 \pm 3.6$ g just before infiltration (Day 3; $n = 6$) and $8.5 \pm 3.6$ g at day 4. Eventually, intradermal infiltration of the nonsteroidal anti-inflammatory drug (NSAID) ketoprofen at 10 mg/kg induced a significant but smaller increase of von Frey threshold (Fig 1B), from $7.7 \pm 2.0$ g before injection (Day 3) to $14.0 \pm 6.5$ g after 30 minutes, and back to $8.5 \pm 3.6$ g after 24 hours (Day 4; $n = 6$).

**Physiological Consequences of the Radiotelemetric Probe Implantation**

One of the major interests of radiotelemetry is the continuous and parallel monitoring of several physiological parameters in nonhandled freely-moving animals. We used this interesting feature to characterize the physiological consequences of our major surgical procedure (Fig 2).

Rats are nocturnal animals, normally active at night and resting during day. They thus display more elevated values of heart rate and body temperature during night than during their diurnal resting period (Fig 2). As shown in Fig 2A, the normal circadian rhythm of alternating high and low values of physiological parameters was strongly altered after surgery. In this representative example (but also seen for all animals monitored), we noted an impaired mobility during night time, reduced
abdominal temperature changes between night and day
time, and long-lasting tachycardia, for about 10 days
after surgery. It should be noted that we did not show
pretest (eg, before surgery) for radiotelemetric studies
because transmitters cannot record parameters before
being implanted. To overcome this difficulty, we used
day 15 as a control, because, from day 15 and for up to
2 months, mean heart rate, mobility, and abdominal
temperature remained stable and were not significantly
different (Fig 2B, see also Fig 3). It should be noted that
postoperative stabilized values were previously shown
to be similar to baseline values, at least for heart rate,
in mice.1

Long-Lasting Mechanical Hyperalgesia
Following Surgery is Prevented by Local
but Not Systemic Perioperative
Ropivacaine Care

In an attempt to reduce recovery time from surgery
and to limit pain symptoms of the implanted rats, we
decided to use ropivacaine during the perioperative
period. In a first group of animals (n = 4), a single subcuta-
neous infiltration of ropivacaine was performed around
the scapula (eg, far from the incision) in order to reveal
a possible systemic effect. Under these conditions, we
failed to reveal any systemic effect on pain symptoms
because mechanical hyperalgesia (Fig 4A) followed the
same time course as saline controls (not shown but see
Fig 1A). We next performed a single intradermal infiltra-
tion of ropivacaine just after surgery and before awaken-
ing of animals (Fig 4B). Surprisingly, this single
infiltration fully prevented the appearance of mechan-
ical hyperalgesia (Fig 4B) after surgery. The initial mean
nociceptive threshold (20.5 ± 6.4 g at day 1; n = 4) was
similar to that observed for naïve animals and for un-
treated rats having fully recovered from surgery (Fig 1).
As seen in Fig 4B, ropivacaine infiltration fully prevented
the appearance of hyperalgesia during the 10 days fol-
lowing surgery, although ropivacaine should have been
fully cleared after a much shorter time (Fig 1B). When
ropivacaine was administered immediately before lap-
arotomy, a similar result was observed (Fig 4C). Measured
with von Frey filaments around the scar, nociceptive
mean threshold value of this group was 22.0 ± 8.0 g at
day 1 (n = 4). It remained stable and not significantly
different during the following days (Fig 4C).

The spectacular effect of perioperative ropivacaine
care on pain symptoms was extremely intriguing because
there is little, if any, consensus regarding the use of local
anesthetics for improving recovery from surgery in hu-
man and very little evidence in animal experimentation.
For all these reasons, we took advantage of our highly
resolutive radiotelemetric monitoring of heart rate,
mobility, and abdominal temperature to objectively characterize postoperative recovery in nonhandled freely-moving animals.

**Perioperative Care With Ropivacaine Improves Recovery From Surgery**

Implanted rats received a perioperative intradermal infiltration of either ropivacaine or saline (9% NaCl) at the end of the surgery. As previously seen (Fig 2; noninjected rats), the normal circadian rhythm of alternating high and low values of physiological parameters was strongly altered after laparotomy in the control group (saline-injected). This is particularly well illustrated in Fig 3A in the form of rhythmograms for a single animal. The diurnal and nocturnal average value of each parameter over 12 hours was also calculated for each animal and displayed in Fig 3C as group means.

During the first nights following surgery, all animals had a reduced mobility. They were mainly active at the beginning and at the end of the night, with recurrent periods of immobility in the interval (Fig 3A, upper panel). This resting period was progressively reduced as the animals recovered. The mobility index for the active period had a low initial mean value of 2.9 ± 1.0 AU (arbitrary units, n = 8) during the night after surgery, and progressively increased to a stable value of 6.8 ± 1.1 AU at day 15 (Fig 3C, upper panel, black circles). Heart rate was elevated immediately after surgery (Figs 3A and C, middle panels), reaching 358 ± 64 bpm and 35.7 ± 0.3°C at day 1, n = 8) than after full recovery (380 ± 26 bpm and 38.15 ± 0.07°C at day 15; Fig 3C, middle and lower panels, black circles). During the diurnal resting period, mobility remained minimal, with an overall average value of 2.7 ± 0.3 AU (Fig 3C, upper panel, open circles). It was never significantly different from the diurnal value at day 15. However, heart rate was elevated immediately after surgery (Figs 3A and C, middle panels), reaching 358 ± 42 bpm at day 6, before decreasing to a stable control value of 322 ± 26 bpm at day 15 (Fig 3C, lower panel, black circles).

**Figure 3.** Consequences of perioperative ropivacaine care on the recovery of mobility heart rate and abdominal temperature. (A, B) Representative rhythmograms of mobility (top), heart rate (middle), and temperature (bottom) in a control (saline-injected: A) and ropivacaine-treated animal (B). In the ropivacaine-treated animal, a regular day/night pattern was established from the first day for each mobility and heart rate while this rhythm was strongly altered in control animals. The pattern of temperature changes was similar in both animals. (C) Average values of recorded parameters over 12 hours for 15 postoperative days. The values are given during the resting period (7–19 hours, light; white symbols) or the active period (19–7 hours, dark; black symbols) in control (n = 8) and ropivacaine-treated rats (n = 8). Three-way ANOVA, with between factor treatment (control versus ropivacaine) and within factors bio-rhythm (night versus day) and days after treatment, showed a significant effect of the interaction of the 3 factors in the case of mobility (F<sub>14, 196</sub> = 3.72, *P* < .001) and of heart rate (F<sub>14, 196</sub> = 3.39, *P* < .001), and, in the case of temperature, no effect of treatment, but an effect of the interaction of the 2 within factors (F<sub>14, 196</sub> = 30.00, *P* < .001). Statistically significant differences between the control group (n = 8) and the ropivacaine-treated group (n = 8) are marked by # for night and * for day values. Dashed horizontal lines show stabilized values (average at day 15).
middle panel, open circles). Diurnal heart rate values were significantly different from that at day 15 during the first 8 days after surgery. The initial recorded values of body temperature were about 33°C ± 14°C, due to the partial loss of homeothermic regulation while animals were still recovering from general anesthesia in their home cage. Although this is not obvious on the temperature rhythmograms (Fig 3A, lower panel), during the first week following surgery the animals displayed a slight diurnal hyperthermia, which was better seen on average values (37.55 ± 30°C at day 2; Fig 3C, lower panel, open circles). This hyperthermia progressively disappeared and abdominal temperature stabilized at 37.24 ± 15°C. Diurnal average temperature was significantly different from the value at day 15 during the first 5 days after surgery, whereas night values were no longer different from the final stabilized value after the 3 first days.

Altogether, full recovery of physiological parameters was achieved after about 8 days in control rats (saline-injected; n = 8; Fig 3) similarly to noninjected animals (n = 4; see Fig 2). As animals recovered their basal day/night rhythm, the difference between the diurnal and the nocturnal values of physiological parameters progressively increased.

In good agreement with the absence of pain symptoms following surgery, with respect to control animals (saline-injected), ropivacaine-treated animals showed less disturbed circadian rhythms for mobility, heart rate, and abdominal temperature, immediately after surgery and during the following days (Fig 3B). Moreover, they did not show reduced nocturnal mobility or diurnal tachycardia, as did control animals. As soon as day 2, these animals exhibited stable values of diurnal heart rate (336 ± 30 bpm; n = 8) and nocturnal mobility (5.6 ± 1.4 AU), similar to those obtained at day 15 for the corresponding parameters (330 ± 20 bpm, and 6.5 ± 0.6 AU respectively) (Fig 3C, upper and middle panels). It should be noted, however, that the initial diurnal hyperthermia, probably resulting from the scarring process, resisted ropivacaine treatment, because both vehicle- and ropivacaine-treated animals displayed the same temperature time course (Fig 3C, lower panel). It should also be noted that we failed to reveal any effect of systemic perioperative ropivacaine (n = 8; not shown) or intradermal ropivacaine at postoperative day 3 (not shown; n = 8) on the recorded physiological parameters.

Taken together, these results show that an immediate postoperative ropivacaine infiltration is able to suppress the durable postoperative hyperalgesia and the associated motor and autonomic manifestations of pain, such as the elevation of diurnal heart rate. We also performed a single infiltration of ropivacaine 10 minutes before starting the surgery. Similarly to what was found in animals treated with ropivacaine after surgery, animals rapidly showed normal circadian rhythms, stable values for nocturnal mobility and diurnal heart rate (not shown, n = 4). In good agreement with the lack of pain signs using radiotelemetry, the mechanical thresholds were not significantly different from naïve animals or from implanted animals having received ropivacaine at the end of the surgery before awakening.
Heart Rate Variability Analysis is a Good Quantitative Indicator of Pain Expression and Confirms the Interest of Perioperative Ropivacaine Care

To reinforce our conclusions, we performed a heart rate variability (HRV) analysis using the high-resolution ECG recorded from our implanted animals (see Methods). HRV changes are indeed widely used to monitor levels of consciousness during anesthesia and elevated HRV are sometimes claimed to be associated with the presence of chronic pain.

We first characterized mean HRV parameters (SD1, SD[SD1], and pNN18) at postoperative day 3 (eg, on hyperalgesic animals), before and after intradermal infiltration of ropivacaine (Fig 5). This treatment was previously shown to transiently suppress abdominal hyperalgesia resulting from the implantatory surgery (Fig 1B). Hyperalgesic rats treated or not with ropivacaine did not show any significant differences in their mean heart rate which remained around 300 bpm (not shown). This was not the case for HRV parameters (Figs 5A–C), which were clearly different between hyperalgesic (Day 3 before injection; Day 4, 24 hours after injection) and nonhyperalgesic rats (Day 3, just after injection; Day 15, control, long after recovery from hyperalgesia). After intradermal infiltration of ropivacaine (Figs 5A–C; black bars) SD1 and SD[SD1] mean value were of 1.78 ± 1.15 seconds and of 0.68 ± 0.16 seconds and statistically smaller than their respective control measured before infiltration (SD1: 3.96 ± 0.72 seconds; SD[SD1]: 2.86 ± 0.43 seconds; n = 4). RR event intervals differing by more than 18 ms were frequently observed in hyperalgesic animals (Day 3: 6.59 ± 1.15%; Day 4: 7.38 ± 2.05%; n = 4) whereas they were extremely rare after ropivacaine treatment (Day 3 after ropivacaine: 1.09 ± 0.85%; n = 4) or after full recovery from surgery (Day 15: 0.49 ± 0.34%; n = 4).

To understand further the effects of ropivacaine in perioperative care, we performed a similar HRV analysis on animals recovering from surgery and having received an intradermal infiltration of ropivacaine (Figs 5D–F, black bars) or saline (Figs 5D–F, white bars) at the end of surgery and before awakening. Saline-treated animals at day 3 exhibited high values for SD1 (3.72 ± 1.07 seconds; n = 8), SD[SD1] (2.76 ± 1.32 seconds; n = 8), and pNN18 (5.39 ± 2.33%; n = 8). These values were significantly reduced ropivacaine-injected rats (Figs 5D–F, black bars; SD1: 1.65 ± 0.17 seconds; SD[SD1]: 0.48 ± 0.12 seconds; pNN18: 0.35 ± 0.23%; n = 8) and similar to those found in nonhyperalgesic rats, eg, having fully recovered from hyperalgesia at day 15.

Altogether, these results strongly suggest that HRV analysis and in particular the quantification of SD1, SD[SD1], and pNN18 are precious quantitative indexes of pain expression. Moreover, they are likely to be useful in order to monitor spontaneous pain expression, at least in this model of abdominal hyperalgesia following surgery.

Discussion

The findings indicate that perioperative analgesia using the long-acting local anesthetic ropivacaine is efficient to prevent postoperative pain following a controlled...
abdominal surgery aimed at implanting a miniaturized radiotelemetric probe in the rat. During the postoperative period we were able to measure the time course of mechanical hyperalgesia and the timely-correlated signs of pain seen as long-lasting alteration of the animal mobility, elevated heart rate, and variability. Hyperalgesia and pain-associated physiological alterations persisted for up to 10 days in our experimental conditions and were fully precluded by a single preventive ropivacaine treatment during the perioperative period (before animal awakening) but not at later stages.

Postoperative pain is a major issue for clinicians, but its basic mechanisms as well as any attempt for its prevention in the long term still need to be clarified. The most widely used model is the incisional paw pain model, but postoperative pain has also been studied after abdominal surgery and skin/muscle incision and retraction, mostly illustrating short-term alterations in the behavior and pain hypersensitivity with variable pharmacological sensitivity to analgesics. Our surgical procedure produced an important mechanical hyperalgesia during the postoperative period, which could be reduced by an acute treatment with ropivacaine, morphine, or ketoprofen in awake animals (Fig 5). This analgesic effect was, however, transient because hyperalgesia was detected 24 hours after the injection of all drugs. Focusing on the properties of local anesthetic action, this time-course is consistent with the half-life of ropivacaine which is of about 2 hours, as characterized in human studies but never in rodents. A similar finding was observed in other models with local anesthetics or other analgesics. During night and daytime, radiotelemetry allowed us to monitor autonomic parameters (electrocardiogram and body temperature) as well as locomotor activity in freely moving animals. This high time resolution monitoring gave us precious complementary information on the postsurgical recovery of the implanted rats. In particular, elevated autonomic parameters, a well-known response to a nociceptive stimulus, were timely correlated with the presence of mechanical hyperalgesia in implanted rats. The mobility impairment also followed the same time course and was accompanied by parallel physiological changes in temperature.

One of the most interesting observations of this work was the effect of a single perioperative injection of ropivacaine, ie, an injection made while the animals were yet under anesthesia, either before the incision or after being sutured. We found that ropivacaine was efficient in removing mechanical hyperalgesia around the scar not only after the immediate awakening of the animal, an expected result, but also during the following days of recovery. The reasons for the long-lasting effect of the local anesthetic ropivacaine are not yet clear. Indeed, pre- and post-incisional hindpaw injection with the local anesthetic bupivacaine was previously shown to be unable to reduce or prevent the development of persistent mechanical hyperalgesia resulting from superficial skin incision. Our work further suggests that ropivacaine perioperative care is efficient in more traumatic surgery involving skin, muscle, and peritoneum incision. At the same time, bupivacaine efficiently reduced dorsal horn neuronal hyperexcitability associated with pain symptoms in awake animals for about 2 hours after incision or at later stages. Here, the long-term efficacy of ropivacaine preventive analgesia is also supported by the monitoring of heart rate and locomotor activity which, in the case of ropivacaine-treated animals, were not significantly different from animals having fully recovered from surgery (>15 days). Interestingly, there is increasing evidence in the human literature for a beneficial long-term effect of ropivacaine treatment, which has been shown sometimes to accelerate postoperative recovery and to limit the use of opioid.

The results obtained in the current study and in the human studies cited above suggest that ropivacaine perioperative analgesia represents an interesting procedure to limit or block the peripheral afferent barrage of nociceptive messages which occurs after anesthesia and gives rise to persistent mechanical hyperalgesia. By preventing this hyperexcitability resulting from the surgical incisions and trauma, ropivacaine might be effective to fully block the establishment of peripheral and central pain sensitization, mechanisms that are known to trigger pathological plastic changes in the nociceptive system. This hypothesis may explain the absence of hyperalgesic phenotypes in implanted rats recovering from surgery (from day 0 to day 15) after perioperative ropivacaine treatment. At the molecular level, central sensitization is thought to be mediated by the establishment of long-term potentiation of glutamatergic sensori-spinal synapses, the duration of which is determined by the intensity of the afferent stimulation. In good agreement, our surgical procedure involves large incisions of skin, abdominal muscles, and peritoneum, performed in a standard time of 50 minutes. This long surgery time was indeed associated to a prolonged postoperative pain period (about 10 days) that was significantly shortened (3 days, not shown) if the same surgery was achieved within 15 minutes. Interestingly, sensori-spinal LTP is mediated, for a large part, by the recruitment of NMDA type glutamate receptors, a mechanism which may be critical for the expression of postoperative hyperalgesia in the incisional paw model. In our model, we used the NMDA antagonist ketamine to anesthetize the animal during probe implantation. It is thus tempting to speculate that perioperative ropivacaine prevents NMDA-mediated LTP during the progressive clearance of ketamine. This point requires further investigation. In any case, it seems that persistent hyperalgesia following incisional surgery needs to be accompanied by a sustained nociceptive stimulation, and this might be the result of a maintained peripheral/central hyperexcitability (not tested here, but see Pogatzki et al) or the uncomfortable mechanical stimulation of the newly implanted radiotelemetric probe.

In conclusion, we show here that ropivacaine preventive analgesia is efficient not only to control immediate postoperative pain, but also to prevent long-lasting alteration of motor and autonomic function. The efficacy of such a preventive analgesia is time dependent and needs to be performed before awakening of the animal because ropivacaine analgesia was only transient at
References


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