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Opioid Induced Loss of Local Anesthetic Potency in the Rat Sciatic Nerve

Qing Liu, M.D., Ph.D.[*] and **Michael S. Gold, Ph.D.**[*,‖]

[*]Department of Anesthesiology, University of Pittsburgh, Pittsburgh, PA, 15213

[‖]Department of Medicine (Division of Gastroenterology Hepatology and Nutrition) and Department of Neurobiology University of Pittsburgh, Pittsburgh, PA 15213 USA

Abstract

Background—Previous evidence suggests that opioid tolerant patients are less responsive to local anesthetics (LAs) for postoperative pain management.

Methods—To determine whether this apparent loss of LA potency is due to an intrinsic change in the peripheral nerve, the effect of systemic morphine was assessed on the potency of lidocaine-induced block of the compound action potential (CAP) in isolated rat sciatic nerves. Analgesic efficacy was assessed with the heat withdrawal assay.

Results—While acute administration of 10mg/kg morphine had no detectable influence on lidocaine potency, seven daily subcutaneous injections of morphine produced a three-fold decrease in potency (EC_{50} for block A- and C- waves for naïve rats were 186 ± 32μM (n = 6, mean ± SD) and 201 ± 31μM (n = 6), respectively; versus 608 ± 53μM, (n = 6) and 613 ± 42 μM, (n = 6), respectively ($p < 0.001$), in nerves from rats that had received seven daily injections of morphine (10 mg/kg)). This loss in potency was both dose- and injection number-dependent, such that the magnitude of the loss of lidocaine potency was significantly ($n=6$, $p < 0.01$) correlated ($r^2 = 0.93$) with the development of morphine tolerance. Interestingly, despite the complete recovery of analgesic efficacy within days following cessation of morphine administration, the morphine-induced decrease in lidocaine potency was fully manifest even 35 days after the last morphine injection. Co-administration of naloxone (1 mg/kg, i.p.), but not naloxone methiodide (1 mg/kg, s.c) with each of seven daily injections of morphine blocked the decrease in lidocaine potency.

Conclusions—These preclinical data suggest that the morphine induced decrease in LA potency is due, at least in part, to the intrinsic changes in the peripheral nerve. Identification of the underlying mechanisms may suggest strategies for more effective post-operative pain management in the growing population of opioid tolerant patients.

Introduction

Peripheral nerve block with local anesthetics (LA) has become an integral part of postoperative pain management enabling better pain control with less opioid use and

Corresponding Author: Michael S. Gold, PhD, University of Pittsburgh Department of Anesthesiology, 3500 Terrace Street Room E1440 BST, Pittsburgh, PA 15213, P: 412-383-5367, F: 412-383-8663, msg22@pitt.edu.

Conflicts of Interests: none

consequently fewer opioid related side effects, more rapid utilization of physical therapy, and a reduction in the length of hospital stay [1,2]. And while it is generally appreciated that post-operative pain management in opioid tolerant patients can be particularly difficult [3], pre-clinical and clinical studies [4-6] suggest that this is not only due to a decrease in the analgesic efficacy of opioids, but may reflect a decrease in the potency of LAs as well. Such a decrease in efficacy not only further limits the therapeutic options available to these patients, but increases the risk of serious LA-related side effects including local and systemic toxicity [7]. Thus, an opioid-induced loss of LA potency may pose a significant clinical challenge, particularly in the face of an increase in opioid use as the first line treatment for perioperative pain.

Given the number of complicating factors that may influence the appearance of a loss of LA potency in opioid tolerant patients, we determined whether intrinsic changes in peripheral nerves could account for such a loss of LA potency in opioid tolerant patients in a rat model of opioid tolerance. Our results suggest that induction of morphine analgesic tolerance was sufficient to drive a three-fold decrease in lidocaine potency, a change that lasted for weeks beyond the last morphine injection, was proportional to the degree of tolerance, and was dependent on central opioid receptor activation.

Methods

Animals

Adult male and female Sprague Dawley rats (275–350 g; Envigo (Harlan Sprague Dawley), Indianapolis, IN) were used for all experiments. Rats were housed in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited animal care facility with unrestricted food and water on a 12:12-hr light-dark cycle (lights on 7AM). All procedures involving animals were reviewed and approved by the University of Pittsburgh Institutional Animal Care and Use Committee (Pittsburgh, PA). Animal care and handling were in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Drugtreatment

All drugs used in the study were purchased from Sigma-Aldrich, St. Louis, MO, and were used at the following the doses: Morphine 3mg/kg, 5mg/kg, or 10mg/kg, s.c., naloxone 1 mg/kg, i.p., naloxone methiodide 1 mg/kg, s.c. Drugs were dissolved in normal saline, which served as a vehicle control. For the experiments to determine the dose-dependence of the morphine-induced changes of LA potency, animals received 7 daily morphine injections (3, 5 or 10 mg/kg s.c, n=6 for each group). For the experiments to determine the onset of the morphine induced changes of LA potency, animals received 3 or 5 daily morphine injections (10 mg/kg, s.c., n=6). For the experiments to determine the extent to which recovery from morphine tolerance was associated with the recovery in the loss of LA potency, animals received 7 daily morphine injections (10 mg/kg, s.c., n=6), and then morphine analgesic efficacy and LA potency were assessed 7, 21, or 35 days after the last morphine injection. Morphine analgesic efficacy was assessed 30 minutes after a subcutaneous injection of morphine based on estimated half-life of morphine analgesia [8]. For the initial 10 mg/kg

experiment, and the onset and dose-dependence experiments, sciatic nerves were harvested 1 day after the last morphine injections. For the recovery experiments lidocaine potency was assessed within 3 hours of the morphine or vehicle injection. For the experiments to determine the role of μ-opioid receptor activation in the loss of LA potency, naloxone (1mg/kg, i.p.) or naloxone methiodide (1mg/kg s.c.) were co-administered with morphine (10 mg/kg) on each of the seven daily injections. Changes in LA potency were assessed one day later. Because experiments were performed in a blinded manner, no formal randomization scheme was employed. However, because rats were pair housed, we attempted to counterbalance which of the two rats in a cage (first or second) received the same drug treatment.

Behavioral testing

Because the primary goal of this study was the characterization of changes in lidocaine potency in the isolated peripheral nerve, behavioral data were only collected as a means to assess the analgesic efficacy of systemic morphine administration. The heat withdrawal assay was used for this purpose because the analgesic response to morphine in this assay is robust and well characterized. Animals were placed in a plexiglass test chamber (diameter 18cm × 22 cm) on a 3-mm-thick glass plate. The glass surface temperature was maintained at 30°C by a feedback-controlled, resistive heating system embedded in the glass (iiTC Life Sciences, Woodland Hills CA). Rats were acclimated to the test chamber for 20–30 min prior to testing. The radiant heat source was from a projection bulb placed directly under the plantar surface of the rat's right hind paw. The paw-withdrawal latency to radiant heat stimulation was defined as the time from onset of radiant heat to withdrawal of the rat's hind paw. The intensity of the radiant heat source was adjusted to result in baseline latencies of 10-12 sec. This was achieved with a heating rate over the first 10 sec of 3°C/sec. A terminal temperature of ~54°C was maintained until cut-off. A cut-off of 20 s was employed to avoid tissue injury. The results from three trials (with an inter-trial interval of 5 min) were averaged to yield a mean withdrawal latency. Tests were conducted 30 min before and after either saline or morphine injection. These settings were consistent with those used previously [9]. The experimenter collecting the behavioral data was blinded to the treatment. However, because the response to 10 mg/kg was so pronounced, it was difficult to maintain blinding in the initial morphine vs vehicle experiments. Nevertheless, blinding was effectively maintained for the dose response, and opioid receptor antagonist experiments.

Recording CAPs from isolated sciatic nerves

Sciatic nerve harvest and CAP recordings were performed as described previously [10]. Briefly, rats were anesthetized with an i.p. injection of 1 ml/kg of a mixture of ketamine (55 mg/ml)/xylazine (5.5 mg/ml)/acepromazine (1.1 mg/ml). The sciatic nerve was removed from ischial tuberosity to tibialperoneal bifurcation (average length = 4.7 cm), then placed in ice cold oxygenated Locke solution (in mM) (136 NaCl, 5.6 KCl, 14.3 $NaHCO_3$, 1.2 NaH_2PO_4, 2.2 $CaCl_2$, 1.2 $MgCl_2$, 11 dextrose, pH = 7.2-7.4). The nerve was then transferred to a recording chamber in which the nerve was submerged in Locke solution with the distal end connected to platinum stimulation electrodes and the central end to a glass suction recording electrode. The distal end of the nerve was separated from the central end with a grease gap. The recording chamber was continuously superfused (2-5 ml/min) with Locke

solution at room temperature with or without lidocaine. The experiments were done at the room temperature because we sought to analyze the impact of lidocaine on A- and C-waves in relative isolation, and this is difficult to achieve at elevated (e.g., body) temperatures, especially with relatively short nerves (<40 mm). The recording at room temperature may have influenced our results, but note that local anesthetic potency at elevated temperatures, at least in our hands, is comparable to that observed at room temperature [10]. The compound action potential (CAP) was evoked with constant voltage electrical pulses of 0.2 to 0.5 millisecond in duration, applied at 0.05 Hz, where the stimulus intensity was ~2 times that needed to evoke a maximal amplitude C-fiber component of the CAP. The stimuli were applied with an electrical stimulator (Model S48, Grass Instruments Co., Middleton, WI). The recording electrode at the other end of the nerve was connected to the input stage of a differential preamplifier (0.1–10 kHz; WPI model DAM-80, Sarasota, Florida). Voltage data were digitized via a CED 1401 Micro A/D converter and analyzed using CED Spike 2 version 5 for MS Windows (CED, Cambridge, England). The average of 6 consecutive CAPs were rectified and integrated to quantify A- and C-fiber components as area under the curve (AUC). The A-fiber deflection of the CAP (A-wave) was distinguished from the C-fiber deflection (C-wave) based on the conduction velocity and amplitude (Fig 1A). After establishing a stable baseline over at least 30 minutes of recording, lidocaine was applied to the nerve at increasing concentrations, where a stable response was achieved prior to applying the next higher concentration (Figure 1B). To control for time-dependent changes in the CAP, vehicle treated nerves were studied over the same time course as required for generation of a complete lidocaine concentration-response curve. As an additional control, the extent to which CAP block reversed following wash of the lidocaine was also determined (Fig 1B). The EC_{50}, or concentration of lidocaine needed to block 50% of the CAP, was used as a measure of potency, and was determined from concentration response curves generated with the application of lidocaine at 30μM, 100μM, 300μM, 1000μM, and 3000 μM. These data were fitted with a Hill equation: $(1 - CAP_{lido}/CAP_{baseline}) = 1/([drug]^{nH} + EC_{50}^{nH})$, where CAP_{lido} is the CAP in the presence of lidocaine, $CAP_{baseline}$ is the CAP prior to the application of lidocaine, and *nH* is the Hill coefficient.

The same experimenter (QL) performed the behavioral and electrophysiology experiments. The experimenter remained blinded to drug treatment until all electrophysiological experiments had been completed.

Data analysis

The number of animals used in this study was determined with a power analysis based on variability previously observed in the study of rat sciatic nerves with local anesthetics [10] and our desire to be able to detect at least a 50% change in potency (EC_{50}) with a power of 0.8 and alpha of 0.05, with four groups analyzed with a one way ANOVA. While our a priori hypothesis was that there would be a decrease in potency, data were nevertheless analyzed with two tailed tests. The Holm-Sidak test was used for post-hoc comparisons. A- and C-waves were analyzed separately. Pearson correlation coefficient was used to determine the association between the morphine induced changes in lidocaine EC_{50} and the morphine analgesic efficacy. The percent maximum possible effect (%MPE) was used to estimate analgesic efficacy, and was calculated according to the formula: %MPE = 100 × (Post-drug

latency – pre-drug baseline latency) / (20 –pre-drug baseline latency). Changes in withdrawal latency over time were analyzed using a mixed design two-way analysis of variance (ANOVA) with treatment as the independent factor and the time as the repeated factor. The Newman-Keuls test was used for post-hoc comparisons between groups. Statistical analysis was performed with SPSS 19.0 statistical software package (SPSS, Chicago, IL, USA). Data are reported as mean ± standard deviation unless otherwise indicated.

Results

Loss of LA potency in morphine treatedrats

Consistent with the results of previous studies, we observed a concentration-dependent block of both A- and C-waves of the sciatic nerve CAP by lidocaine (Fig. 1C, D). EC_{50} for lidocaine-induced block of the sciatic nerves from naïve rats were 186 ± 32 μM (n = 6) and 201 ± 30 μM (n = 6), for the A- and C-waves, respectively. In nerves from rats that received seven daily injections of morphine (10 mg/kg s.c.) there was a significant ($p < 0.01$) decrease in lidocaine potency for both A- (608 ± 53 μM, n = 6) and C- (613± 42 μM, n = 6) waves, respectively (Fig 1 C, D). All animals started in this and subsequent experiments were retained through to the CAP analysis. Consequently, it was not necessary to implement a strategy for dealing with missing data during statistical analysis.

Morphine-induced loss of LA potency is dose dependent and present in females as well as males

We next sought to determine whether the decrease in LA potency was dose-dependent. Two additional groups of rats were studied that received seven daily injections of three (n = 6) or five (n = 6) mg/kg morphine. Because female rats are more sensitive to morphine than males [11], two groups of female rats were studied to determine whether there was also a sex difference in the impact of morphine on LA potency. The female rats received seven daily injections of either vehicle or five mg/kg morphine. To determine the analgesic efficacy of the morphine administered, heat withdrawal latencies were determined before and 30 min after morphine administration. The first morphine injection was associated with a significant ($p < 0.01$) increase in withdrawal latency to a duration close to cut-off (~20 s) in all rats tested regardless of dose or sex (Fig 2 A). However, the morphine-induced increase in withdrawal latency decreased in each group with subsequent morphine injections such that there was a significant ($p = 0.025$) loss of analgesic efficacy in each group, by day seven, relative to the increase in latency observed on day one (Fig 2 A). The withdrawal latency observed in the presence of morphine on day seven was also significantly different ($p = 0.031$) between male groups treated with morphine, with the longest latency associated with the three mg/kg dose and the shortest latency associated with the 10 mg/kg dose (Fig 2 A). Furthermore, while the withdrawal latency in male rats receiving three mg/kg and five mg/kg were still significantly longer than that either baseline withdrawal latency or saline treated rats, the withdrawal latency in rats receiving 10 mg/kg, was no different ($p = 0.264$) from baseline or saline-treated rats. Finally, when withdrawal latency were analyzed as a function of sex and time with female rats compared to male rats receiving either five or 10 mg/kg, there was a main effect associated with time ($p < 0.01$), but no significant influence

of sex ($p = 0.067$), or a significant interaction between sex and time. Thus, the development of tolerance was comparable in males and females.

Lidocaine potency was tested on nerves from each group of rats the day after the last injection. There was a significant ($p < 0.01$) decrease in lidocaine potency (increase in EC_{50}), in all nerves from morphine treated rats relative to that on nerves from naïve or saline treated rats for both A- (Fig 2B) and C- (Fig 2C) waves. Furthermore, the morphine-induced decrease in lidocaine potency was dose-dependent in male rats, such that the greatest decrease in potency was observed in the 10 mg/kg group and the smallest in the three mg/kg group (Fig 2B and C). The decrease in lidocaine potency in female rats receiving five mg/kg morphine, was not significantly ($p = 0.261$) different than the decrease in potency in male rats receiving five mg/kg morphine, but significantly ($p =0.024$) less than the decrease in male rats receiving 10 mg/kg morphine for both A- and C-waves. (Fig 2B and C).

Onset of morphine-induced loss of LA potency

To determine the timing over which the morphine-induced loss of LA developed, two additional groups of male rats were studied, receiving three ($n = 6$) or five ($n = 6$) daily injections of 10 mg/kg. s.c. morphine. The morphine-induced increase in paw withdrawal latency in these two groups mirrored that observed in the group receiving seven daily injections, with the maximum increase in latency observed on day one, and a smaller increase in latency observed with each subsequent injection (Fig 3A). The increase in latency observed after three daily injections was significantly greater than baseline, but significantly smaller than the increase observed on day one (Fig 3A). The same was true after five daily injections. However, the increase in latency after the fifth injection in the five day group was also significantly less than that after the third injection in the three day group.

There was a significant decrease in the potency of lidocaine-induced block of both the A- (Fig 3B) and C- (Fig 3C) waves in sciatic nerves from rats receiving three and five daily injections of morphine, relative to that in saline-treated and naïve rats. However, the magnitude of the decrease in potency was injection number-dependent, where the decrease was greatest after seven daily injections, and the smallest after only three daily injections. The differences in potency between each group were also significant ($p < 0.01$, Fig 3B and C).

Correlation between morphine tolerance and the loss of LA potency

To explore the relationship between the development of morphine tolerance and the loss of LA potency, we analyzed both dose-dependence (3, 5 and 10 mg/kg) and onset (3, 5 and 7 daily injections) analgesia data as a function of the shift in LA potency. To estimate tolerance, paw withdrawal latency data were transformed to a % of the maximal possible effect (%MPE), where an increase in latency to cut-off was considered full analgesic efficacy and the absence of a morphine-induced change in latency was considered complete tolerance. EC_{50} data were then plotted relative to the %MPE (Fig 4). The association between %MPE and EC_{50} was linear and highly significant with an $r^2 = -0.92$, $p<0.01$ for A wave and -0.93, $p<0.01$ for C wave, where a 50% decrease in analgesic efficacy was associated with a more than 100% decrease in lidocaine potency.

Recovery of morphine-induced loss of LA potency

We next sought to determine the time course over which the morphine-induced loss of LA potency recovers following cessation of morphine administration and to determine the extent to which recovery from tolerance was associated with the recovery in the loss of LA potency. To address these issues, additional groups of rats were first treated with seven daily injections of morphine (10 mg/kg), and then the morphine analgesic efficacy and the LA potency were assessed seven (n = 6), 21 (n = 4), or 35 (n = 5) days after the last morphine injection. Consistent with the first group of rats tested after seven daily injections of morphine, there was no change in paw-withdrawal latency after the morphine injection on day seven relative to baseline. Furthermore, seven, 21 or 35 days after the last morphine injection, there was no behavioral evidence of tolerance, as morphine-induced increase in paw withdrawal latency was comparable ($p = 0.173$) to that observed in rats that received their first morphine injections (Fig 5 A).

The potency of lidocaine-induced block of A- and C-waves in sciatic nerves from saline-treated rats challenged with morphine hours before nerves were harvested was comparable to that observed in nerves from naïve rats (Fig 5B and 5C). However, the decrease in lidocaine potency observed in all groups of morphine treated rats was significant ($p < 0.01$) compared to that in saline treated or naïve nerves (Fig 5B, 5C). Furthermore, there is was no evidence of a recovery of the decrease in potency, even after 35 days, as the EC_{50} in the nerves from this group was comparable ($p = 0.304$) to that tested the day after seven daily injections of morphine (Fig 5B, 5C). This was observed for both A and C waves.

Morphine induced loss of LA potency is blocked by naloxone but not naloxone methiodide

To determine whether the morphine-induced loss of LA potency is due to the activation of μ-opioid receptors, and if so, whether these receptors are located in the periphery, four additional groups of animals were studied. These included rats receiving seven daily injections of naloxone 1 mg/kg, i.p., or naloxone methiodide 1 mg/kg, s.c alone, or each of these antagonists co-administered with morphine (10 mg/kg, s.c. n = 6 per group). Naloxone would block both central and peripheral opioid receptors, whereas the blood brain barrier impermeant analog of naloxone, naloxone methiodide, would only block peripheral opioid receptors. As expected, neither naloxone, nor naloxone methiodide had any detectable influence on paw withdrawal latency ($p =0.168$, Fig 6A). Similarly, there was no detectable ($p =0.072$) increase in paw withdrawal latency in rats treated with the combination of naloxone and morphine. However, there was a significant increase in paw withdrawal latency in rats co-administered naloxone methiodide and morphine (Fig 6A).

The potency of lidocaine-induced block of the A- (Fig 6B) and C- (Fig 6C) waves of the CAP in sciatic nerves from rats treated with naloxone or naloxone methiodide alone, was comparable ($p =0.085$) to that observed in nerves from naïve rats. There was also no change ($p =0.163$) in lidocaine potency in nerves from rats treated with the combination of naloxone and morphine (Fig 6B and C). However, there was a significant decrease in lidocaine potency in nerves from rats treated with the combination of naloxone methiodide and morphine relative or data from naïve rats, or from the other three groups in this experiment (Fig 6B and C). Furthermore, the lidocaine potency in nerves from the rats treated with

naloxone methiodide and morphine was comparable ($p = 0.173$) to that on nerves from rats treated with morphine alone (Fig 6B and C).

Discussion

The purpose of this study was to determine whether morphine tolerance is associated with a loss of LA potency in isolated peripheral nerves. Repeated systemic morphine administration resulted in a decrease in LA potency that was comparable in males and females. This decrease in potency was dependent on the dose of morphine and the number of injections, and consequently, was significantly correlated with the development of tolerance to morphine-induced analgesia. Strikingly, the loss of LA potency persisted beyond the recovery of tolerance. Lastly, the loss of LA potency was blocked by naloxone, but not naloxone methiodide. These results suggest the apparent decrease in LA potency observed in opioid tolerant patients, most clearly documented by Hashemian and colleagues [5], may be due, at least in part, to a decrease in the potency of LA-induced peripheral nerve block.

While there are a number of potential mechanisms that could account for the observed decrease in LA potency, we suggest that our experimental design involving an isolated nerve continuously superfused over virtually its entire length, argues most compellingly for a mechanism that involves changes intrinsic to the nerve. The use of the isolated nerve eliminated possible changes in blood flow which could affect lidocaine clearance. Similarly, bathing the whole nerve in lidocaine not only minimized the potential impact of changes in drug access to its target, but eliminated changes in myelination and / or the density or distribution of ion channels (e.g. Na^+ and K^+ channels) that can affect charge spread within an axon, and consequently potency estimates based on focal application of lidocaine. That changes in both K^+ and Na^+ channel expression have been documented in response to repeated morphine injection [12,13] suggests we may have under estimated the impact of morphine administration on the apparent decrease in LA potency that would be observed with focal LA administration.

While nociceptive testing was necessary to establish the association between the development of analgesic tolerance and the decrease in LA potency, the nociceptive testing necessarily introduced a potential confound: that it was not the morphine, but the repeated heating of the hindpaw responsible for the changes in both A- and C-fibers. However, arguing against this possibility are 1) a single injection of morphine, which was the only manipulation in which animals were consistently stimulated to cut-off, was not associated with a decrease in LA potency; 2) the decrease in potency was most robustly manifest in rats in which complete tolerance had been generated, a state in which the rats were responding to the thermal stimuli at the same latency observed in naïve rats, 3) there was no evidence of persistent sensitization in animals that had been driven to cut-off, as withdrawal latencies were the same the day after morphine as they were at baseline; and 4) the stimulation paradigm including baseline latency, cut-off, number of stimuli employed to establish baseline, and the inter-stimulus interval, were comparable to those used extensively be other investigators with no evidence of persistent sensitization.

The primary target of LAs is voltage gated Na^+ channels (VGSCs). LA are thought to access a low affinity site in channels in the resting or closed state, but a high affinity site(s) in channels in open and inactivated states [14]. Consequently, any processes that lead to an increase in the fraction of channels residing in the low-affinity state will result in a decrease in LA potency. These processes include:1) a hyperpolarization of the membrane potential (relieving the high affinity state associated with channel inactivation), 2) a post-translation-induced change in the biophysical properties of the channels, such that more channels reside in the low affinity state, and 3) a change in expression of VGSC subunits such that there is either an increase in the expression of channels with an intrinsically lower affinity for LAs or in channels with different biophysical properties, such that there is a shift in the fraction of channels residing in low vs high affinity states [15]. Among these changes, the latter two phenomena have been more clearly documented. For example, the VGSC beta subunits can influence both fast and slow inactivation, with changes in the phosphorylation of the channel influencing these properties as well[16]. Similarly, the tetrodotoxin (TTX)-resistant Na^+ channel (Nav1.8), is intrinsically less sensitive to local anesthetic block, than TTX-sensitive channels in DRG neurons[17]. Ongoing studies are designed to distinguish which, if any of these possibilities contribute to the morphine-induced decrease in LA potency.

While both the dose-dependence and the time-course of the morphine-induced decrease in LA potency suggest that the induction of analgesic tolerance is necessary for the loss of LA potency, these two phenomena may be mechanistically unrelated. Morphine clearly produces a number of changes in the body in addition to the suppression of nociceptive signaling, and the differential time course and/or magnitude of the tolerance that develops in response to repeated morphine administration for several of these changes, suggests that underlying mechanisms are distinct [18]. Furthermore, it is unlikely that any of the synaptic mechanisms that have been implicated in the development of analgesic tolerance, such as the activation of NMDA receptors [19] or the induction of long term potentiation (LTP) [20] contribute directly to the morphine-induced decrease in LA potency, because even if due to presynaptic changes, additional mechanisms would be needed to account for the decrease in LA potency observed in both A- and C-fibers. Similarly, the observation that the morphine-induced decrease in LA potency is blocked by naloxone, but not naloxone methiodide argues against any of the previously described changes in the primary afferent such as the increase in TRPV1, ERK, c-Jun, and p38MAPK [21], that have been suggested to contribute to the development of analgesic tolerance. However, while the same signaling molecules mediate both analgesic tolerance and the decrease in LA potency remains to be determined, the morphine induced activation of cells in the dorsal horn, such as microglia [22,23], that are able to release mediators capable of acting back on primary afferents could contribute to the development of analgesic tolerance and the decrease in LA potency in both A- and C-fibers.

A striking finding from the current study was that the morphine induced decrease in LA potency persisted for 35 days after the last morphine injection. Minimally, the observation that the morphine-induced decrease in LA potency outlasted analgesic tolerance indicates that while these two phenomena may share a common induction mechanism, the mechanisms controlling the persistence of these changes are distinct. There are at least two general mechanisms that could account for the persistence of the decrease in LA potency in primary afferents. First, there is a persistent change in a cell in the CNS, such as a microglial

cell in the dorsal horn that enables the cell to maintain the change in primary afferents. Consistent with this possibility, there is evidence of a repeated morphine-induced increase in histone acetylation in the spinal cord [24]. Identification of the signaling molecules mediating the maintenance of the change in primary afferents would suggest a way to restore LA potency.

A second mechanism to account for the persistent change LA potency would be a persistent change in primary afferents secondary to mediators released in the CNS during the repeated morphine administration. Persistent changes in nociceptive afferents have been described in response to a single exposure of inflammatory mediators such as PGE_2 [25], NGF, TNF-alpha, orIL-6 [25,26], and most recently, the mu-opioid receptor agonist, DAMGO [27]. These previously described changes are associated with a prolonged hyperalgesia in response a subsequent inflammatory challenge, rather than the shift in LA potency. Nevertheless, similar to the results obtained in the present study, the persistent changes in nociceptive afferents were not associated with overt changes in nociceptive behavior, and were only manifest upon subsequent challenge. While our naloxone results argue against a role for peripheral mu-opioid receptor activation in the loss of LA potency, it will be interesting to determine whether any of the mechanisms implicated in the prolonged hyperalgesic response contribute to the induction and/or maintenance of the decrease in LA potency.

While we focused on the peripheral nerve in the present study, there are at least two observations suggesting that the changes observed in the peripheral nerve may develop in the central nervous system as well. There is evidence that the duration of anesthesia achieved with intrathecal administration of 5% lidocaine is significantly shorter in opium abusers [4]. Similarly, the analgesia associated with a single intrathecal injection of lidocaine is decreased in morphine tolerant rats [6]. And while a change in the central axons of primary afferents may contribute to these previous results, it will be interesting to determine whether resistance to LAs develops in dorsal horn neurons, or at even higher centers in the CNS, potentially pointing to a deleterious interaction between opioids and other use-dependent Na^+ channel blockers used for the treatment of pain.

Our results with 3 mg/kg morphine are likely to have the greatest clinical significance, because they suggest that even a moderate dose of morphine that did not produce complete tolerance produced a significant decrease in LA potency. This suggests that there may be a decrease in LA potency even in patients on moderate doses of opioids. The result may be the need for higher LA infusion rates in these patients. Minimally, this suggests that standardized post-operative pain management protocols [28] would have to be adjusted for this population in order to achieve optimal post-op pain management. However, such an increase LA administration would also be associated with an increase in the risk of LA toxicity. Furthermore, because the decrease in LA potency is persistent, it should not be sufficient to simply get patients off opioids prior to surgery. Rather, it will be necessary to identify mechanisms underlying the shift or more relevantly its maintenance, so as to be able to reverse them as a way of restoring LA potency. In the meantime, our results suggest that other analgesic strategies than peripheral nerve block should be pursued for opioid tolerant patients.

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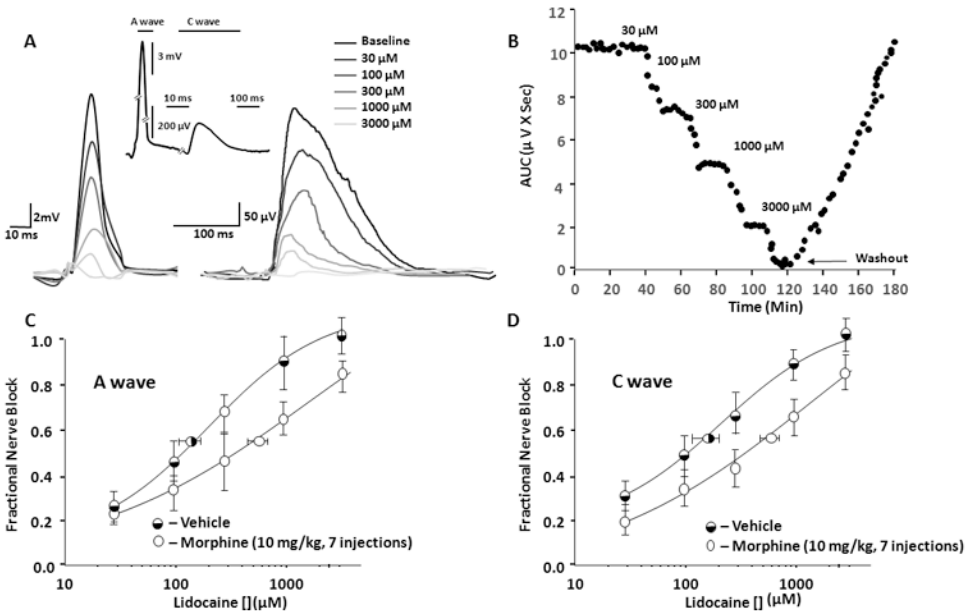

Figure 1.

A. Representative traces of the compound action potential (CAP) recorded from an isolated sciatic nerve from a naïve rat. Traces are of the A- and C- waves before and after increasing concentrations of lidocaine were applied. Inset: An example of a raw CAPwaveform before rectification. **B**. Diary-plot of the C-wave area under the curve (AUC) illustrating time and lidocaine concentration dependent changes in the AUC. Pooled concentration-response curve data for A- (**C**) and C-(**D**) waves of the CAP evoked in nerves from naïve and morphine treated (10 mg/kg s.c., for seven days) male rats. Fractional nerve block is calculated as (1- AUC_{drug})/$AUC_{control}$. In this and subsequent figures, pooled data are plotted as mean ± SD (n = 6 for each group). Mean EC_{50} values are plotted with horizontal error bars. In this and subsequent figures, significant differences between groups over time and/or dose as revealed through post-hoc comparisons have been omitted for clarity.

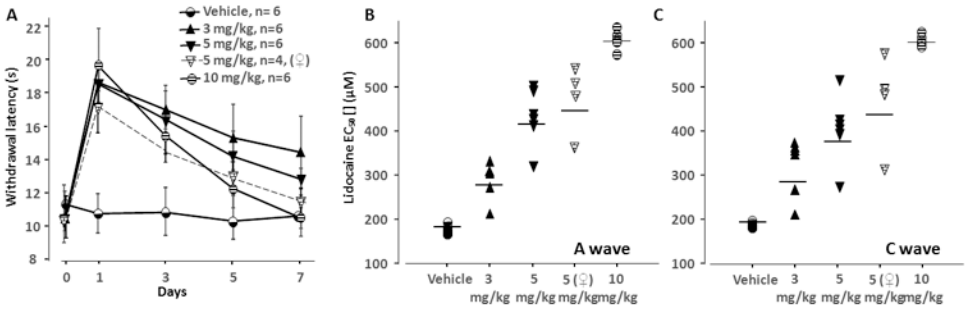

Figure 2.

A. Changes in paw withdrawal latencies before and after daily injections of morphine to male (3mg/kg, 5 mg/kg, or 10 mg/kg) and female (5 mg/kg) rats. Lidocaine EC_{50} data for block of A- (**B**) and C- (**C**) waves evoked in sciatic nerves obtained from the rats whose behavioral data are plotted in A. Each point represents data from a single rat and the bars are the mean EC_{50}.

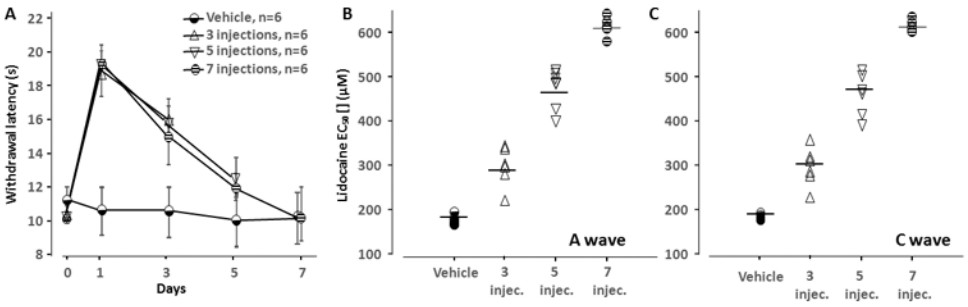

Figure 3.

A. Changes in paw withdrawal latencies before and after three, five and seven daily morphine injections of morphine (10 mg/kg, s.c.) to male rats. Lidocaine EC_{50} data for block of A- (**B**) and C- (**C**) waves evoked in sciatic nerves obtained from the rats whose behavioral data are plotted in A. Each point represents data from a single rat and the bars are the mean EC_{50}. Data from the seven daily injection group plotted in Figure 2 have been replotted here for comparison.

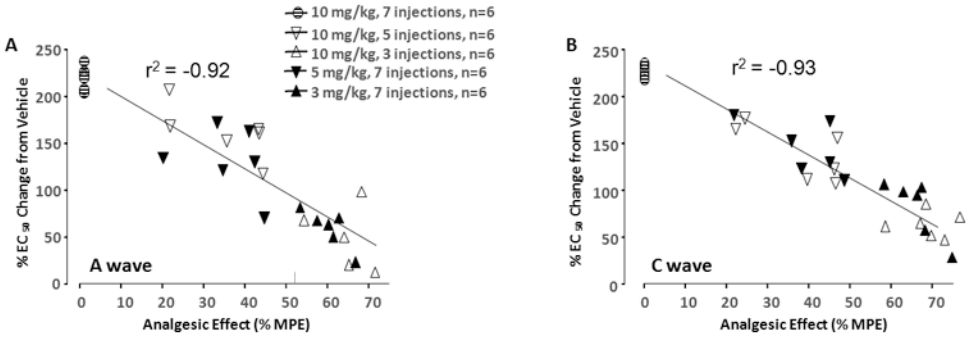

Figure 4.

Paw withdrawal data calculated as a percent of the maximal possible effect (% MPE) are plotted as a function of the change in EC_{50} for the lidocaine-induced block of the A- (**A**) and C- (**B**) wave of the sciatic nerve CAP, relative to the mean EC_{50} observed in nerves from naïve rats. Data are from the rats treated with morphine at different dose and/or different amounts of time, illustrated in Figures 2 and 3. The relationship between %MPE and EC_{50} was linear and highly significant ($p < 0.01$) for both A- and C-waves.

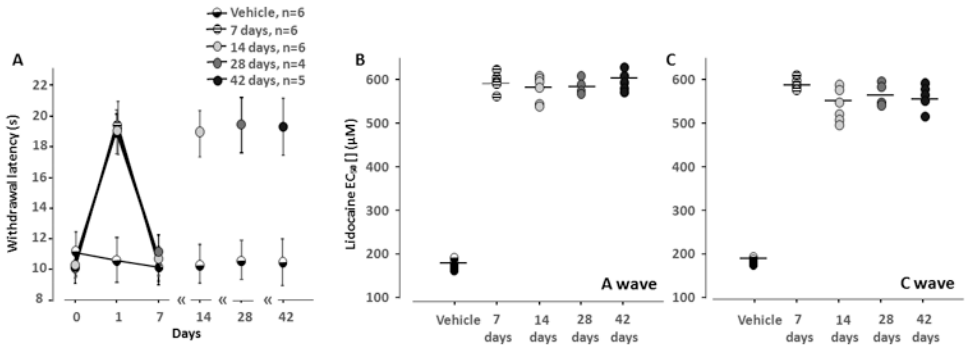

Figure 5.

A. Changes in paw withdrawal latencies before and then after seven daily morphine (10mg/kg) injections, as well as seven, 21 and 35 days after the last morphine injection. Full recovery of morphine analgesic efficacy was achieved seven days after the last morphine injection. Lidocaine EC_{50} data for block of A- (**B**) and C- (**C**) waves evoked in sciatic nerves obtained from rats whose behavioral data are plotted in A. Each point represents data from a single rat and the bars are the mean EC_{50}. There was no significant ($p > 0.05$) difference between morphine treated groups with respect to EC_{50} for A- and C-waves, but all morphine treated groups was significantly ($p < 0.01$) higher than that in vehicle.

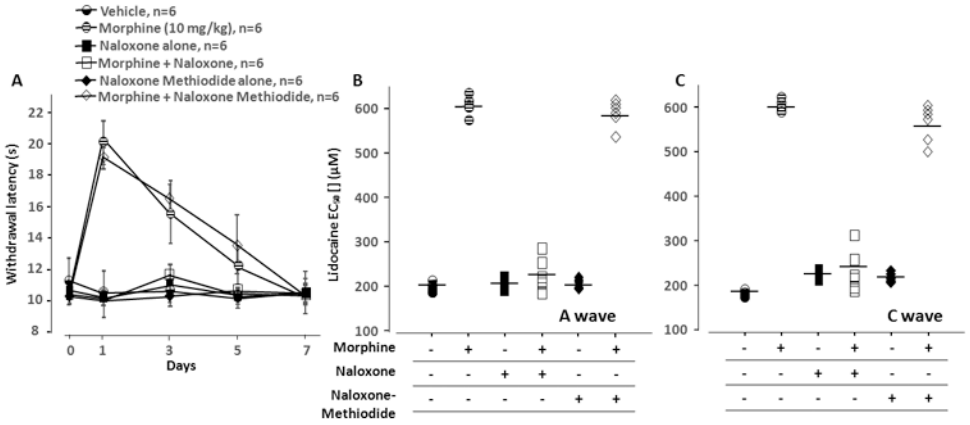

Figure 6.

A. Changes in paw withdrawal latency before and then after seven daily injections of morphine (10 mg/kg), naloxone (1mg/kg, i.p.), naloxone methiodide (1mg/kg s.c), or the combination of morphine (10 mg/kg) with naloxone (1 mg/kg) or naloxone methiodide (1 mg/kg). Data from vehicle treated rats have been replotted for comparison. Lidocaine EC_{50} data for block of A- (**B**) and C- (**C**) waves evoked in sciatic nerves obtained from the rats whose behavioral data are plotted in A. Each point represents data from a single rat and the bars are the mean EC_{50}. There was no significant difference between vehicle, naloxone, naloxone methiodide, or naloxone + morphine treated groups with respect to EC_{50} for A- and C-waves. Nor was there a significant difference between morphine and morphine + naloxone methiodide treated groups with respect to EC_{50}. However, the EC_{50} for both morphine and morphine + naloxone methiodide treated groups were significantly greater than that in all other groups for both A- and C-waves.