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Chronic inflammatory pain does not attenuate the development of tolerance to chronic morphine in adult male rats

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ABSTRACT

The overall impact of chronic pain on the response to opioids is ambiguous in the literature, and comparisons between human and animal studies are complicated by vast differences between the manner and dosage of opioids given to humans treated for pain in comparison to rodents as well as a lack of healthy participant studies examining the impact of chronic opioids. The purpose of this study was to evaluate the impact of chronic pain on the development of tolerance to morphine and to assess how the concentration of drug affects this process. Twenty-four hours after the injection of CFA or normal saline in the left hind paw, the level of mechanical hypersensitivity was assessed and animals were randomly assigned to a morphine dose (1, 3 or 8 mg/kg or saline). Morphine was administered by subcutaneous injection twice a day for 5 days. On Day 6, animals were challenged with a single dose of 3 mg/kg morphine prior to formalin testing. Evidence of tolerance was mixed, and the results varied widely among the conditions. Analysis of mean paw withdrawal thresholds indicated that the analgesic efficacy of subcutaneous morphine diminished following repeated dosing. The presence of the chronic inflammatory pain condition during the morphine dosing period produced an increase in formalin pain behaviors compared to saline controls, such that animals given any dose of morphine during the 5-day dosing period showed higher responding to formalin following the 3 mg/kg dose than animals that had received saline injections. These results indicate that chronic pain does influence the development of opioid tolerance, but it does not prevent this phenomenon from occurring as suggested by some researchers.

1. Introduction

Opioids remain one of the most commonly prescribed analgesics for post-operative, post-injury, and chronic pain conditions, such as back pain, fibromyalgia, and migraines (Chou et al., 2009). Among the undesirable side effects reported by patients on opioid therapy, one of the biggest concerns for practitioners is the development of tolerance during chronic treatment. Patients who no longer experience the full analgesic effect of opioids may begin to hoard pills or seek out additional prescriptions in order to maintain an acceptable level of pain relief (Bell and Salmon, 2009). The psychological impact of tolerance is significant as well, to the point that pain relief is only secondary to the addictive, rewarding properties of the drug. Exploring how this process is influenced by chronic pain could lead to better treatment programs that help alleviate the cycle of tolerance and drug seeking. Tolerance to opioids can lead to forms of hyperalgesia, which complicates treatment even further (Hay et al., 2008); however, there is also evidence that the stress from chronic pain may attenuate the development of tolerance to morphine (Vaccarino et al., 1993, 1997; Vaccarino and Couret, 1993, 1995; Yu et al., 1997; Davoudi et al., 2005).

The overall impact of chronic pain on the response to opioids is ambiguous in the literature, and comparisons between human and animal studies are complicated by vast differences between the manner and dosage of opioids given to humans treated for pain in comparison to rodents as well as a lack of healthy participant studies examining the impact of chronic opioids (Petersen et al., 2008). The purpose of this study was to evaluate the impact of chronic pain on the development of tolerance to morphine and to assess how the concentration of drug affects this process. We hypothesized that chronic pain (monoarthritis modeled by the injection of an inflammatory agent) would attenuate the development of morphine tolerance, based on previous studies suggesting that the presence of pain reduces tolerance and modifies drug-seeking behavior in rodents (Colpaert et al., 1980; Vaccarino et al., 1993; Lyness et al., 1989; Zollner et al., 2008). We also hypothesized that there would be a dose-dependent relationship between the concentration of morphine administered and the level of tolerance present. Further, an interaction between pain condition and dose was anticipated,

Abbreviations: MPWT, mean paw withdrawal thresholds; CFA, complete Freund's adjuvant; SC, subcutaneous.

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with normal (non-chronic pain) animals receiving the highest concentration of morphine expected to demonstrate the highest levels of tolerance.

2. Methods and materials

2.1. Subjects

Seventy-four adult male Sprague–Dawley rats (4–9 per group), weighing between 200 and 300 g, from the University of Texas at Arlington vivarium were used in this project. Animals were housed in groups of 3–4 and maintained on a 12-hour light/dark cycle (lights on 7:00 a.m.). Approval was obtained from the University of Texas at Arlington Institutional Animal Care and Use Committee, and all animals were treated in accordance with the guidelines set forth by the International Association for the Study of Pain (Zimmerman, 1983) and the European Commission's Directive 86/609/EEC regarding the care and treatment of experimental animals.

2.2. Drugs

2.2.1. Morphine sulfate

In order to more closely model a chronic pain patient on opioids, we employed systemic injections twice daily at levels that did not impair normal functioning and enabled experimenters to perform behavioral tests on subjects. We elected to use a low, medium, and high dose of morphine (1, 3, and 8 mg/kg, respectively) administered subcutaneously (SC). Doses within this range have produced analgesia in our laboratory previously (Uhelski and Fuchs, 2009). Although oral and intravenous doses are more commonly used in clinical settings, subcutaneous injections are used more often in animal studies because of the ease of administration.

2.2.2. Complete Freund's adjuvant (CFA)

The presence or absence of a chronic inflammatory pain state was established using injections of complete Freund's adjuvant (CFA), while control animals received normal saline, respectively. The injection of CFA into the plantar hind paw induces a chronic inflammatory condition, resulting in mechanical hypersensitivity and severe inflammation of the hind paw area lasting up to several weeks after the injection. Animals that receive these injections guard and/or favor the paw, demonstrate lower mechanical and thermal thresholds, and will avoid preferred areas to escape stimulation of the affected area (LaBuda and Fuchs, 2000; Boyce-Rustay et al., 2010; Cook and Moore, 2006; Xu and Huang, 2002). This indicates that the injected area is allodynic and normal stimuli are bothersome. Previous research has indicated that mechanical thresholds can be restored to normal levels in CFA-injected animals that are administered analgesics such as aspirin or morphine (Liang et al., 2006), and so this condition was used to emulate that of a chronic pain patient.

2.3. Design and procedure

2.3.1. Behavioral testing

Mechanical hypersensitivity was evaluated by assessing paw withdrawal thresholds to establish the overall sensitivity of the animal to a mechanical stimulus. Animals were habituated to a Plexiglas chamber ($20\times10.5\times40.5$ cm) on top of a mesh screen for 15 min. The size of the chamber allowed for free movement of the animal and the mesh screen allowed for the application of calibrated von Frey monofilaments to the plantar surface of the left and right hind paw. Paw withdrawal thresholds were acquired using the up/down technique (Dixon, 1980) with eight von Frey monofilaments (3.85, 5.68, 9.74, 18.39, 39.42, 77.3, 135.3, and 251.34 mN). Each trial began with the 1-second application of a 10 mN von Frey, and if no response was detected than the next highest force was applied. If there was a response (withdrawal of the paw), then the next lowest force was applied. This procedure was repeated until either no response was made at the highest force, or there had been 4 von Frey stimuli applied following the initial response. Three trials were conducted, and the scores were averaged across trials to determine mean paw withdrawal thresholds (MPWT) for the left and right paw of each animal.

Withdrawal thresholds for each paw in a given trial were calculated using the following formula: $[Xth]\log = [vFr]\log + ky$, where [vFr] is the force of the last von Frey used, $k = 0.2593$ which is the average interval (in log units) between the von Frey monofilaments, and y is a value that depends upon the pattern of withdrawal responses. This method used for calculating the withdrawal threshold for each trial is based on Dixon's (1980) method for determining threshold values when the outcome is all or none. The original design was for LD-50 (in which case the outcome was death and the sequence of stimuli were incrementally increasing doses of a given drug), but this can also be applied to any sensory assessment for which a dichotomous response is obtained. In this case, there is a logarithmic increase in force for each sequential stimulus applied (referred to as k), and each set of von Frey monofilaments is calibrated to confirm this relationship. The pattern used to determine the value of "y" in the paw withdrawal assessment consists of up to five responses, starting with a positive response (X) followed by either no response (O) or additional positive responses (X). For each pattern, "y" was determined on a table based on the number of stimulations that did not elicit a response before the first paw withdrawal (1–4). According to the table produced by Dixon (1980), a response pattern of XOXOX following 2 non-responses would produce a y value of -0.458, which is then added to the equation above to calculate a threshold score. This value is a pre-determined maximum likelihood estimates for each possible pattern of responses based on the assumption that response thresholds form a normal cumulative distribution. For the example given, $\log(Xth) = \log(36.42\text{ mN}) + (.2593^{*} - 0.458)$; $Xth = 29.99$ mN. For animals that do not respond to the highest von Frey, $y = 1.00$ and the threshold value is the inverse log of $\log(251.34\text{ mN}) + (0.2593^{*}1.00)$, or 456.63. This method enables us to base the sequence of stimuli on the animal's response for increased accuracy.

The level of tolerance was evaluated using the formalin test, which utilizes the rapid, intense inflammation produced by subcutaneous injection of dilute formaldehyde. All animals, regardless of condition, received a 3 mg/kg challenge dose of morphine 30 min before the formalin test. Animals that had developed tolerance to the morphine were expected to demonstrate higher levels of pain compared to those that had received saline only, representing a loss of analgesic effect following repeated doses. Following an injection of 0.05 ml 1% formalin into the plantar right hind paw, animals were placed in a Plexiglas observation chamber ($30\times30\times30$ cm) with mirrors angled below to allow the experimenter to view the hind paws without disturbing the subject. The number of seconds the animal spent licking the paw, elevating the paw, and resting the paw on the floor surface was recorded utilizing toggle-key software for each of the three behavior states. Paw down described the animals' state with the formalin-injected hind paw resting on the floor of the chamber, with weight applied normally. Paw elevation included periods during which the animal held its hind paw above the floor, in either a guarding position or with its weight fully resting on the contralateral hind paw. Paw licking consisted of the animal, licking, biting, shaking and/or pulling at the nails of the formalin hind paw. This three-level behavioral assessment of the formalin test has been used extensively in previous research (LaBuda et al., 2001; Donahue et al., 2001; Fuchs et al., 1999).

A weighted formalin pain score was calculated based on the following formula: [0(time spent paw down) + 1(time spent elevating paw) + 2(time spent licking paw)]/300 (seconds). Scores were

calculated for every 5 min, and averaged together for the acute (0–20 min) and tonic (25–45 min) phases of the test.

2.3.2. Experimental procedure

On the first day, animals were habituated to the mechanical threshold testing chamber and baseline values were established to eliminate animals with pre-existing sensitivities to mechanical stimuli. Less than 5% of animals demonstrated baseline thresholds below the maximum value, and none were excluded due to mechanical hypersensitivity. Animals were then randomly assigned to a pain condition. Animals in the chronic pain condition received a subcutaneous injection of 0.15 ml complete Freund's adjuvant (CFA) mixed in equal parts with normal saline into the left plantar hind paw, while animals in the no chronic pain condition received equivalent injections of normal saline only. All injections were performed while the animals were under brief anesthesia (inhaled IsoFlurane) and their activities were monitored during recovery.

Twenty-four hours following CFA injections, animals were again tested for MPWT, to ensure that CFA injections induced an inflammatory state and that saline injections did not alter thresholds. Any CFA animals that did not demonstrate at least a 50% decrease in threshold values were not included in the remainder of the study. Only two animals were eliminated due to MPWT prior to dosing, one from each condition (3% of total subjects).

Animals that met this criterion were randomly assigned to one of four dosing conditions: normal saline, 1 mg/kg morphine, 3 mg/kg morphine, or 8 mg/kg morphine, administered subcutaneously. Experimenters were blind to dosing conditions, but not pain condition. Injections were given at a volume of 1 ml/kg at 9:00 am and 2:00 pm each day for 5 consecutive days. Two hours following the afternoon injection (4:00 pm), MPWT were assessed. Animals treated with saline as opposed to CFA were not expected to demonstrate any changes in mechanical hypersensitivity following morphine treatment, but were assessed daily to ensure that the experimental procedure remained consistent for all animals.

On the sixth day, animals were challenged with a dose of 3 mg/kg morphine 30 min prior to a 0.05 ml subcutaneous injection of dilute formaldehyde (1.0%) in the right hind paw. Following formalin injection, animals were placed in the chamber and behavioral observations were made for 45 min. In addition, a separate group of animals ($n=11$) was tested using the same procedure with chronic saline injections, only the 3 mg/kg challenge dose was eliminated and a saline injection was administered 30 min prior to the formalin test. This enabled us to evaluate whether the presence of CFA-induced paw inflammation would alter the behavioral response to formalin, as CFA-injected rats tend to guard and/or favor the inflamed paw.

3. Results

3.1. Impact of chronic morphine on mean paw withdrawal thresholds (MPWT)

Prior to experimental manipulations, normal animals do not respond to the highest von Frey force applied and are therefore assigned the maximum threshold value. Thus, when animals are tested at baseline or are assigned to saline control groups, there is little variance in threshold scores. For the current study, all animals received repeated MPWT testing for consistency among all subjects. Only 2.4% of animals responded at baseline, and none were excluded due to the 50% criterion for hypersensitivity. MPWT scores for non-CFA animals did not differ from baseline at any time point (data not shown) and were therefore not included in further analyses. In addition, right paw values did not differ from baseline values at any point (data not shown) and were therefore excluded from further analyses. In order to compensate for the baseline MPWT ceiling effect, threshold scores were converted to percent change from baseline for pre-injection and post-injection Days 1 and 5, using the formula % change = (Maximum Score − Individual Score)/Maximum Score. Although MPWT were evaluated daily following dosing, only the first and last days' change scores were analyzed, to compare the analgesic effect acutely to that after chronic dosing and at the time point when any changes elicited by the development of tolerance would be maximal. Since change scores were calculated from baseline thresholds, higher values indicate the presence of mechanical hypersensitivity, and lower values indicate thresholds that are closer to normal. Thus, the injection of an effective analgesic should produce scores closer to zero.

Repeated measures ANOVA was performed to evaluate differences in MPWT percent change scores (dose as the between subject variable; pre-injection and post-injection Days 1 and 5 as dependent variables). Post hoc tests (Fisher's LSD) were performed for significant overall effects. There was a significant main effect for dose ($F_{3,29}=3.47$, $p<0.05$), and time ($F_{2,58}=15.45$, $p<0.001$) but not a significant dose by time interaction ($F_{6,58}=1.65$, ns). All animals in the CFA condition demonstrated hypersensitivity and had at least a 50% change in MPWT from baseline to pre-injection. There were no differences in scores for saline-dosed animals on Day 1 or Day 5 compared to pre-injection, indicating that mechanical hypersensitivity remained consistent in the non-treated control group.

Animals receiving 3 mg/kg and 8 mg/kg morphine had MPWT change scores that were significantly lower than pre-injection scores on Day 1, indicating that these doses produced significant analgesic effects (which elevated threshold scores closer to baseline values). Only animals receiving 8 mg/kg had significantly lower change scores from pre-injection on Day 5 of testing, and the effect was diminished relative to Day 1. Dosing with 1 mg/kg did not produce reliable analgesia at either time point. These results indicate that repeated dosing led to a reduction in the analgesic efficacy of morphine (see Fig. 1A).

For animals receiving only saline treatment on Days 1–5 and 30 min prior to the formalin test, percent changes in MPWT scores from baseline to pre-injection, Day 1 and Day 5 were examined for both CFA- and saline-injected animals using repeated measures ANOVA. There was an overall main effect for group ($F_{1,9}=122.66$, $p<0.001$) but not a significant effect for time ($F_{2,18}=1.61$, ns) or group by time interaction ($F_{2,18}=0.07$, ns). Post hoc analyses (Fisher's LSD) revealed that CFA-injected rats had significantly higher percent change scores relative to saline-injected rats at all three time points (see Fig. 1B).

3.2. Impact of chronic morphine dosing and pain condition on the response to formalin

Differences in response to the 3 mg/kg challenge formalin test were evaluated with both chronic pain (CFA-injected) and no chronic pain (saline-injected) groups. Analysis of formalin test data was performed on pain scores (calculated for every 5 min on a scale of 0–2, with higher scores representing more pain behaviors) that were averaged over the two phases, acute (0–20 min) and tonic (25–45 min). A mixed design repeated measures ANOVA was performed, with group (chronic pain or no chronic pain) and dose (1, 3, or 8 mg/kg morphine or saline controls) as between-subjects variables and mean formalin pain score for each phase (acute or tonic) as dependent variables. There was a significant overall effect for time ($F_{1,55}=156.43$, $p<0.001$) and dose ($F_{3,55}=10.93$, $p<0.001$), as well as a significant dose by pain group interaction ($F_{3,55}=3.26$, $p<0.05$) and dose by time interaction ($F_{3,55}=5.92$, $p<0.005$). There was not a significant main effect for pain group alone ($F_{1,55}<1$, ns), and no other interaction effects were present (F values<3, ns). Thus, post hoc analyses (Fisher's LSD) were performed on the dose by time interaction for each pain group separately.

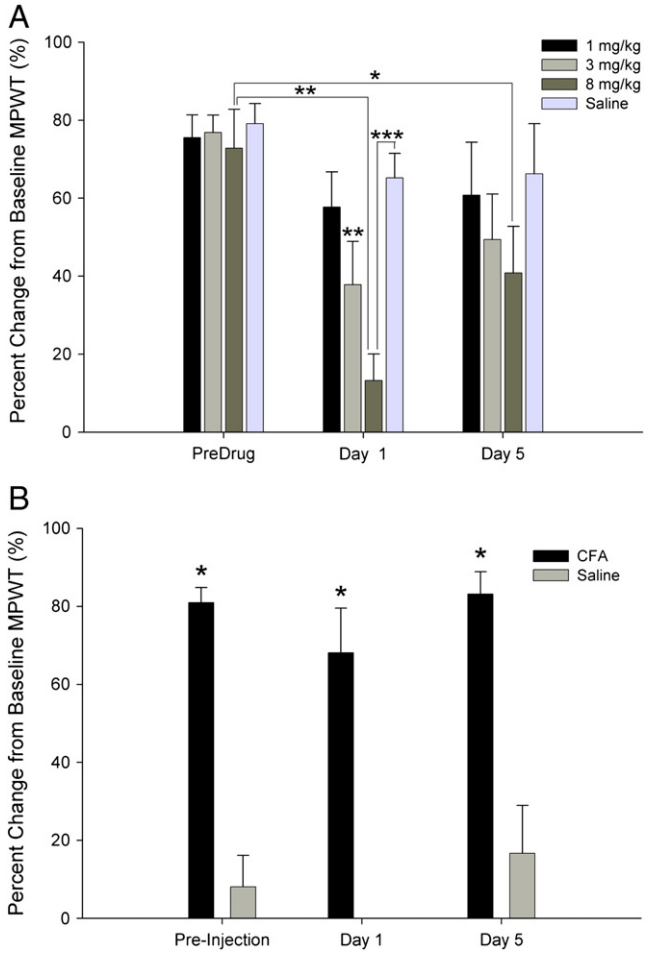

Fig. 1. (A) Mean paw withdrawal threshold change scores for CFA-injected animals. For the CFA group, MPWT percent change scores (expressed as mean ± SEM) indicated the presence of mechanical hypersensitivity at pre-injection testing. The administration of 8 mg/kg morphine resulted in significantly lower percent change scores on Day 1 compared to pre-injection and saline-dosed animals, indicating the analgesic effect of this dose. Day 5 scores were significantly different from pre-injection but did not differ from saline, indicating that the effect has lessened over the course of 5 days of injections. For animals that received 3 mg/kg, scores on Day 1 were significantly different from pre-injection but not significantly different from saline, while Day 5 scores did not differ from pre-injection. The administration of 1 mg/kg or saline did not produce any significant changes in scores compared to pre-injection. $^{***}p = 0.001$ compared to saline $^{**}p < 0.005$ compared to pre-injection $^{*}p < 0.05$ compared to pre-injection.(B) Mean paw withdrawal threshold change scores for saline-dosed control animals. MPWT percent change scores (expressed as mean ± SEM) revealed that animals injected with CFA in the left hind paw demonstrated significantly lower MPWT at pre-injection, Day 1 and Day 5 relative to saline-injected animals. $^{*}p < 0.005$

In the acute phase, chronic pain animals treated with 1 mg/kg and 8 mg/kg demonstrated significantly higher pain scores on average than saline-treated controls. For the no chronic pain group, only animals treated with 8 mg/kg had significantly higher pain scores than saline-dosed controls. For the tonic phase, chronic pain animals treated with any dose of morphine had significantly higher pain scores than saline-dosed controls. For the no chronic pain group, only animals treated with 3 mg/kg demonstrated higher pain scores than animals dosed with saline (see Fig. 2).

Overall, saline-dosed animals in the chronic pain group responded to the 3 mg/kg challenge similar to other opioid-naive animals, with significantly lower pain scores than those seen in normal animals in response to formalin (Oluyomi et al., 1992). However, animals in the no chronic pain group revealed pain scores that were only slightly below normal, suggesting that the analgesic effect of the 3 mg/kg morphine was not as strong in these animals. This indicates that the presence of an inflammatory pain condition has a significant impact on the physiological response to morphine, but there was not an attenuation of the development of tolerance as predicted.

For the saline-dosed control groups, data for CFA- and saline-injected animals were analyzed together. Formalin pain scores were collapsed into acute (0–20) and tonic (25–45) phases and evaluated using repeated measures ANOVA. There was not a main effect for group ($F_{1,9} = 0.36$, ns) or a group by time interaction ($F_{1,9} = 0.39$, ns), but there was an overall main effect for time ($F_{1,9} = 191.22$, $p < 0.001$), indicating that subjects had significantly higher pain scores during the tonic phase of the formalin test. These results confirm that the presence of CFA-induced inflammation did not alter the pattern of formalin-induced pain behaviors (see Fig. 2C).

4. Discussion

The purpose of the current study was to evaluate the impact of a chronic pain condition on the development of morphine tolerance in adult male rats. Paw withdrawal threshold data demonstrated that the analgesic efficacy of morphine decreased with repeated dosing, indicating that some of the morphine-dosed animals developed tolerance over the 5-day dosing period. Unfortunately, the behavioral test used to evaluate these changes could not be used to detect any changes in thresholds present in the no chronic pain group due to the ceiling effect produced when all non-responding animals are assigned the highest possible value. Future studies could employ behavioral tests that allow for both upward and downward shifts, such as the Hargreaves thermal test (Hargreaves et al., 1988) or tail-flick test.

Based on the formalin test analyses, the presence of the chronic pain state significantly altered the response to the 3 mg/kg challenge, but it was in the opposite direction that was predicted. In the chronic pain group, animals receiving 8 mg/kg had significantly higher formalin pain scores during the acute phase than all other groups. In addition, all animals which received chronic morphine over the 5-day period had significantly higher formalin pain scores during the tonic phase of the test than animals which received only saline injections. This pattern was not present in the no chronic pain group, where 8 mg/kg treated animals had significantly higher pain scores compared only to saline-treated animals, and only 3 mg/kg animals had significantly higher formalin pain scores relative to saline-treated animals during the tonic phase. Thus, there was a dose-dependent effect present during the acute phase for both groups and the tonic phase for the no chronic pain group. Part of the reason for this was the fact that the saline-dosed group did not demonstrate adequate morphine analgesia following the 3 mg/kg challenge, and their formalin pain scores were only slightly lower than normal. Differences produced by chronic morphine dosing may not have been statistically significant in comparison to saline-dosed animals but may have still been elevated above normal. The saline-dosed animals may have become habituated to repeated injections with no effect, but since this pattern did not emerge in the chronic pain group, it is unlikely that this is the case.

Our hypothesis predicting that the presence of a chronic pain state would attenuate the development of morphine tolerance was not supported; all doses of chronic morphine produced significantly higher formalin pain scores than saline in response to the 3 mg/kg challenge in the chronic pain group. Morphine tolerance was also present in the no chronic pain group, but the pattern of scores was different for these animals and 3 mg/kg dosing produced the highest formalin pain scores. Thus, our interaction hypothesis was also not supported because the 8 mg/kg no chronic pain animals did not demonstrate the highest formalin pain scores. There were no significant differences among any of the morphine doses for the chronic pain group, which indicates that any level of morphine was capable of producing tolerance over a 5-day dosing period. Therefore, our dose-dependent hypothesis was only partially supported by

evidence from the no chronic pain group indicating that only 3 mg/kg produced a significant tolerance effect.

The presence of the inflammatory pain condition altered the animals' response to opioids, but not in the manner that we predicted. Perhaps the differences detected occurred due to the activity of endogenous opioids released in response to chronic stressors, including pain (Appelbaum and Holtzman, 1986; Vaccarino et al., 1997). Studies examining the receptor changes that follow opioid exposure found that endogenous opioids induce changes that initiate the development of tolerance (such as receptor desensitization and internalization) more rapidly and efficiently than some exogenous opioids, such as morphine (Martini and Whistler, 2007; Koch and Hollt, 2008; Zollner et al., 2008). Although receptor internalization may reflect normal cellular maintenance, which involves removing de-sensitized receptors for phosphorylation prior to returning them to the cell membrane, it may also indicate progress towards down-regulation of receptors (He et al., 2002). The fact that the relationship between receptor internalization and tolerance is not clear and varies depending on the specific agonist suggests that the factors influencing the development of tolerance at the cellular level are more complex than previously suggested.

Another possibility is that putting weight onto the CFA-injected paw during the formalin test may have created another source of unpleasantness, shifting focus from the formalin-injected paw. It was noted during the formalin testing that observers had to monitor the CFA animals to ensure that they recorded only the paw-licking behavior directed at the formalin paw, because they would occasionally switch over to the opposite paw. However, a direct comparison of CFA and saline-injected rats demonstrated that there is no difference in formalin pain scores due to the presence of CFA alone.

Although some animal literature suggests that the presence of pain prevents or diminishes the development of tolerance to repeated morphine dosing (Colpaert et al., 1980; Vaccarino et al., 1993, 1997; Lyness et al., 1989; Zollner et al., 2008), there is also evidence which suggests that the opposite is true (Gutstein et al., 1995; Yu et al., 1997; Liang et al., 2006). Because the conditions, doses, and length of dosing used vary considerably among these studies, it is difficult to draw definitive conclusions about the relationship between chronic pain and opioid tolerance. In addition, some clinical studies have demonstrated that chronic pain patients do not develop tolerance to opioids, or that the effect is minimal and a non-issue in some cases (Twycross, 1988; Chu et al., 2006; Angst et al., 2009). Such ambiguous results suggest that this issue is more complex and necessitates further study to assess the underlying physiological changes that occur during chronic opioid treatment in the presence of a chronic pain state.

The complexity of factors affecting morphine tolerance and analgesia further emphasizes the need for careful examination of methodology when making clinical implications for chronic opioid treatments. In a recent issue of *Pain* and an older issue of *APS Bulletin*, editorials concerning opioid tolerance studies discussed the inherent difficulty in drawing conclusions from animal research for human applications as well as the issue of how to define tolerance in research settings—while animal studies can demonstrate tolerance via a rightward shift in the dose–response curve, clinical tolerance is often identified as a reduction in analgesic efficacy of a constant dose over time (Simonnet, 2009; Cleary and Backonja, 1996). These concepts may be complementary, but are not always consistent. Research in chronic pain patients relies heavily on verbal self-report of pain levels, and results can be obscured by the potent placebo effect and participant bias (Simonnet, 2009).

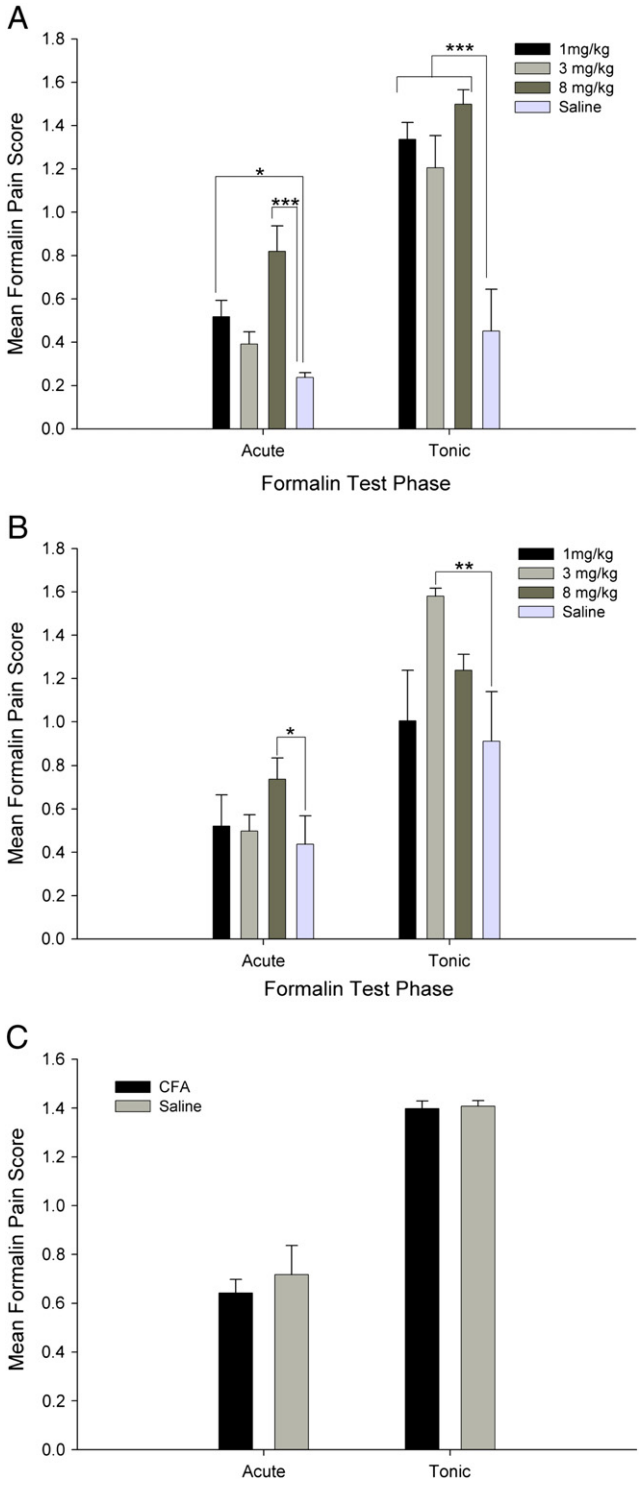

Fig. 2. Weighted pain scores from the formalin test following challenge dose. Formalin test pain scores (expressed as mean ± SEM) following the 3 mg/kg challenge were assessed separately for each pain group. (A) Among the chronic pain animals, saline-dosed animals demonstrated consistent morphine analgesia, with low overall pain scores compared to the morphine-dosed animals in both phases. For the acute phase, saline-dosed animals had significantly lower pain scores than those dosed with 1 mg/kg and 8 mg/kg morphine, and the 8 mg/kg animals had scores that were significantly higher than both 1 mg/kg and 3 mg/kg as well. For the tonic phase, saline-dosed animals had significantly lower pain scores than all three morphine-dosed groups, which did not differ from one another. (B) For the no chronic pain animals, saline-dosed animals failed to demonstrate adequate morphine analgesia to the 3 mg/kg challenge. During the acute phase, only animals dosed with 8 mg/kg had significantly higher pain scores than saline-treated animals. For the tonic phase, only animals that were dosed with 3 mg/kg chronically had significantly higher formalin pain scores compared to saline and 1 mg/kg treated animals.(C) Weighted pain scores from the formalin test following saline dosing only. CFA-injected animals dosed with saline 30 min prior to the formalin test did not demonstrate significantly different formalin pain scores relative to saline-injected rats during either phase of the test. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.005$.

Whether the presence of a pain disorder alters the development and nature of physiological tolerance to opioids is an issue of significant consequence for both the medical community and those patients suffering from such conditions. Inconclusive or contradictory studies make it difficult for those in the medical community to discern whether the potential costs of developing tolerance are balanced by the relief provided by opiates, and many health care providers elect to prescribe them only as a last resort (Cleary and Backonja, 1996), leaving chronic pain patients who could benefit from carefully monitored opioid therapy unable to find adequate relief (Bell and Salmon, 2009). Further research and the development of more clinically relevant models of morphine tolerance and addiction could reduce the impact of this undesirable side effect on chronic opioid treatment.

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