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Intrathecal Catheterization Influences Tolerance to Chronic Morphine in Rats

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We evaluated the antinociceptive effects of acute and chronic morphine administered spinally via lumbar puncture in intrathecally catheterized and sham-surgery rats. The effects of acute morphine did not differ between groups. Catheterized rats developed tolerance to chronic morphine more rapidly, compared with sham and naive rats. Therefore, catheter presence facilitated development of opioid antinociceptive tolerance. Spinal astrogliosis, determined by measurement of 3-dimensional cell volumes, was observed in catheterized rats as indicated by significantly larger cell volumes compared with surgery-naive controls. Gliosis induced by chronic intrathecal morphine administered to surgery-naive animals was comparable to that observed in saline-treated catheterized rats. (Anesth Analg 2012;114:690–3)

Intrathecal catheters have been extensively used to investigate the spinal effects of opioid-induced analgesia and mechanisms of opioid tolerance.[1–6] Most rodent studies used polyethylene tubing for chronically indwelling spinal catheters. DeLeo et al.[7] demonstrated that this intrathecal catheterization induced spinal gliosis. Recent evidence demonstrated that immune cell activation is important in the etiology of morphine tolerance.[8–11] Thus, the effects of catheterization-induced gliosis on acute and chronic morphine tolerance were investigated.

METHODS

Experiments, complying with Canadian Council on Animal Care policies, were approved by the Queen's University Animal Care Committee. Male Sprague-Dawley rats (200–250 g) were housed individually with ad libitum access to food and water, and maintained on a reverse 12/12-hour light/dark cycle.

Indwelling intrathecal catheters (7.5 cm, PE10) were implanted as previously described.[12] A surgical sham involving skin incision and muscle dissection, without breakage of the arachnoid membrane to cause leakage of cerebral spinal fluid was deemed appropriate to control for any postoperative pain. All animals were allowed to recover from surgery for 5 days before testing.

Drugs were injected intrathecally via catheter[13] or lumbar puncture (LP) as previously described.[8] Treatments were administered via either catheters (10 μL) or LP (15 μL). The tail-flick test was used to evaluate thermal nociception (2- to 3-second baseline responses, cutoff time of 10 seconds). All behavioral testing was performed blind to drug treatments.

Immunohistochemistry

Spinal cords were isolated and lumbar segments transected (40 μm) after cardiac perfusion with 4% paraformaldehyde. Free-floating sections were incubated in blocking solution, followed by overnight incubation at 4°C with antisera recognizing glial fibrillary acidic protein (1:2500; DakoCytomation, Mississauga, ON, Canada) to label astrocytes. Sections were incubated with a secondary antibody conjugated to an Alexa 488 fluorophore.

Imaging of immunoreactive cells was performed as previously described.[14] Briefly, immunofluorescence was captured using the Leica TCS SP2 multi-photon confocal microscope (Leica Microsystems, Buffalo Grove, IL). Serial images were captured at 100× magnification, at 0.75-μm increments throughout the z plane (4 series per section, 3 sections per animal). Images were stacked and reconstructed in 3 dimension using ImagePro Plus v5.0 software (MediaCybernetics, Bethesda, MD). Total cell volume was calculated for each cell. All data were collected by an experimenter blind to drug treatments.

Effect of Intrathecal Catheterization on Acute and Chronic Morphine Antinociception

Animals were divided into 3 groups: catheterized, sham-operated, or surgery-naive. After recovery, animals were injected intrathecally with a 50% effective dose of morphine (0.25 μg) (Sabex, Canada) via LP. Nociceptive responses were measured before and after injection for 2 hours. Animals received morphine (1 μg) via LP on days 2 to 5, every 24 hours. On day 6, nociceptive responses were measured prior to morphine injection (0.5 μg) and 30 minutes after drug injection.

Catheter- and Morphine-Induced Astrogliosis

Animals were divided into 2 groups: catheterized or surgery-naive. After recovery, rats were further divided into 4 groups receiving either vehicle (saline) or morphine (15 μg) via catheter or LP injection, once daily for 5 days. Spinal cords were collected on day 6 (see below) and volumes of 3-dimensional astrocytes were measured to determine hypertrophy, an indicator of astrogliosis.

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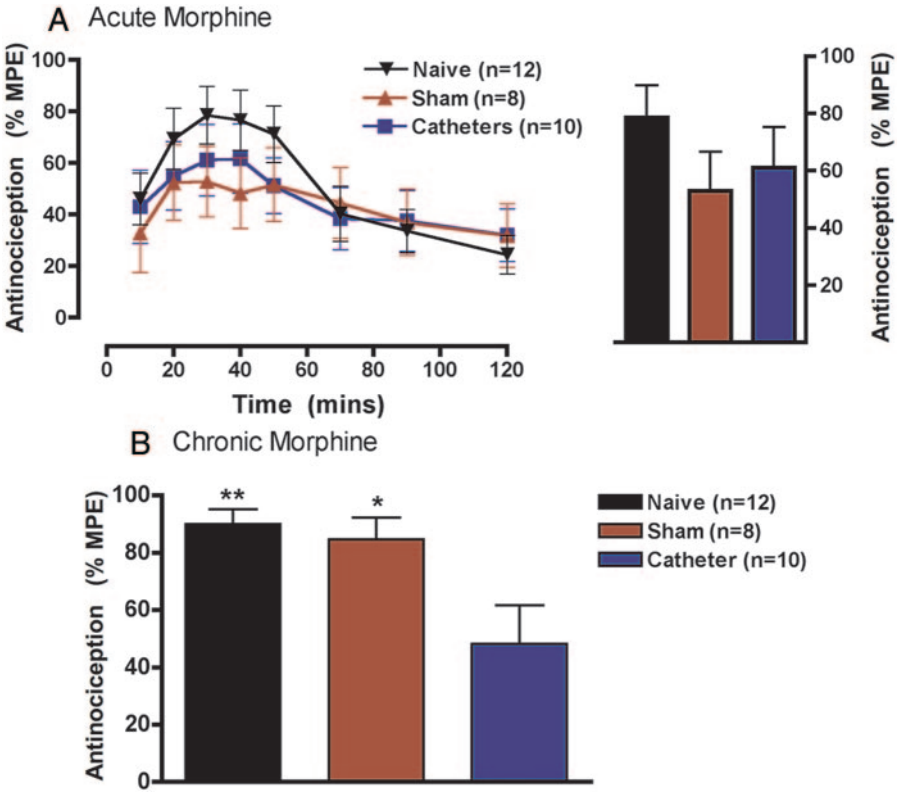

Figure 1. Intrathecal catheterization modulates chronic but not acute morphine effects. A, Catheterized, sham-operated, or surgery-naive animals received intrathecal morphine (0.25 μg) via lumbar puncture. Nociception was measured before and for 2 hours after drug injection. No significant difference was observed between groups. A 2-way repeated-measures analysis of variance (ANOVA) revealed a significant effect of time ($F_{2,189} = 18.89$, $P < 0.0001$), but not of treatment ($F_{2,189} = 0.271$, $P = 0.764$). No significant difference between treatment groups was found using a 1-way ANOVA of the mean areas under the curve ($F_{2,25} = 1.024$, $P = 0.3739$). The mean percentage of maximum possible effect (%MPE) at 30 minutes after morphine is presented on the right. A 1-way ANOVA revealed no significance between treatments ($F_{2,27} = 1.063$, $P = 0.359$). B, After chronic administration of intrathecal morphine (1 μg daily via lumbar puncture for 4 days), animals received intrathecal morphine (0.5 μg) via lumbar puncture. Nociception was measured before and 30 minutes after drug injection. Data are presented as mean ± SEM. A 1-way ANOVA revealed significance between treatments ($F_{2,27} = 6.186$, $P < 0.01$). *Significant difference from catheterized rats; *$P < 0.05$, **$P < 0.01$.

Statistical Analysis

Tail-flick latencies were converted to a maximum possible effect: (postdrug latency − baseline)/(cutoff latency − baseline) × 100. All data are expressed as mean ± SEM. Statistical significance was determined using a 1- or 2-way repeated-measures analysis of variance, followed by Tukey or Bonferroni post hoc multiple comparisons test to determine between group differences. Mean area under the curve was determined for data presented in Figure 1A and analyzed for statistical significance by 1-way analysis of variance. P values <0.05 were considered significant.

RESULTS

Catheterization or sham surgery did not affect the duration or peak antinociceptive effect elicited by a single morphine (0.25 μg) injection compared with nonsurgical rats (Fig. 1A). The mean antinociceptive effects of intrathecal morphine (0.5 μg) injected via LP on day 6, in catheterized, sham-operated, or surgery-naive rats are represented in Figure 1B. Significantly greater analgesia was observed in sham-operated and surgery-naive rats compared with catheterized rats. No difference in antinociception was observed between sham-operated and surgery-naive animals.

Mean volumes of astrocytes from rats treated with chronic vehicle (saline) or morphine (15 μg) via catheter or LP delivery are represented in Figure 2. Astrocytes reconstructed from animals administered saline via catheter showed significant hypertrophy, indicated by increased cell volume, compared with those receiving saline via LP. Astrocytes from animals receiving chronic morphine via LP were significantly larger compared with the LP saline group ($P < 0.001$), but were not different from those receiving chronic saline via catheter ($P > 0.05$). Astrocytes from animals receiving chronic morphine via catheter were significantly larger than those from animals receiving morphine via LP ($P < 0.001$) or those treated with saline via catheter ($P < 0.01$).

CONCLUSIONS

In previous reports, the onset of analgesic tolerance was more rapid in rats administered chronic morphine via intrathecal catheter[6] compared with those receiving morphine via LP.[8] In this study, we demonstrated that catheterization had no effect on acute morphine antinociception but augmented the analgesic tolerance to chronic morphine compared with sham or surgery-naive controls. A submaximal morphine dose was selected that would permit the detection of subtle differences in analgesic responses between groups. Similarly, Wu et al.[15] reported no difference in antinociception between catheterized and noncatheterized mice to acute morphine administration. Interestingly, Prado[16] reported decreased morphine potency in rats with intrathecal catheters inserted by the Yaksh and Rudy method[12] compared with those acutely catheterized via LP. A number of methodological differences may contribute to the differing results, including difference in rat strain, differences in protocols of tail-flick nociceptive testing (heated coil versus radiant light), and differences in route of morphine administration (via acute or indwelling catheter versus LP). Almeida et al.[17] demonstrated hyperalgesia after spinal sensitization by the administration of noxious treatments in rats with indwelling catheters. Therefore, prior sensitization, such as chronic morphine exposure,

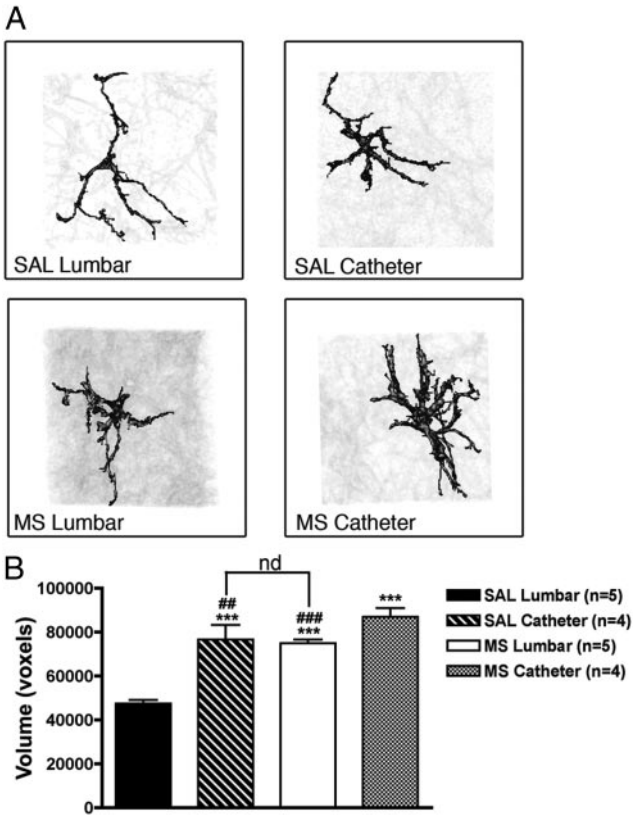

Figure 2. Intrathecal catheterization induced astrogliosis comparable to that induced by chronic morphine via lumbar puncture. A, Representative 3-dimensional images of astrocytes from rats receiving chronic intrathecal saline (SAL) or morphine (15 μg; MS) via catheter or lumbar puncture once daily for 5 days. B, Astrocyte cell volumes were measured. Data are presented as mean ± SEM. A 1-way analysis of variance revealed significance between treatments ($F_{3,433} = 49.96$, $P < 0.001$). *Significant difference from rats administered saline by LP; ***$P < 0.001$. #Significant difference from rats administered morphine by catheter; ##$P < 0.01$, ###$P < 0.001$.

may be required to observe the effects of catheterization on changes in morphine-induced nociception.

Others have reported decreased antinociceptive potency of somatostatin[18] and dynorphin A[19,20] after catheterization surgery. The induction of spinal gliosis by intrathecal catheterization, in combination with that induced by repeated morphine, may oppose the analgesic effects, thereby contributing to the opioid-tolerant state. Our data suggest that catheter-induced inflammation does not alter sensitivity to acute morphine, but enhances the development of tolerance. Astrocyte volumes in morphine-tolerant animals receiving treatment via LP were comparable to those of opioid-naive, catheterized animals. Therefore, the gliosis observed in catheterized and morphine-tolerant animals, despite being comparable in magnitude, may be induced by independent mechanisms. When reviewing the literature in which treatments are administered via intrathecal catheters, potential implications should be considered: catheterization can induce significant morphologic changes in spinal processes,[21] and thus alter nociceptive responses,[17] drug potency, and the onset or the extent of tolerance to repeated drug administration.[15,16]

DISCLOSURES

Name: Theresa-Alexandra Mattioli, BSc.
Contribution: This author helped design the study, conduct the study, analyze the data, and prepare the manuscript.
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Contribution: This author helped conduct the study.
Name: Brian Milne, MSc, MD.
Contribution: This author helped design the study and prepare the manuscript.
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Contribution: This author helped design the study.
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Contribution: This author helped design the study, conduct the study, and prepare the manuscript.
This manuscript was handled by: Steven L. Shafer, MD.

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Validation of Statistical Methods to Compare Cancellation Rates on the Day of Surgery: Erratum

The appendix of a 2005 paper gives steps for iteratively calculating the inverse of the Freeman-Tukey transform.[1] Step 1 should have used the harmonic mean sample size among 4-week periods, not the total sample size.[2] Differences in cancellation rates will be small (e.g., $< 0.1\%$ using the numbers in Table 8 in the article), because sample sizes are large for benchmarking cancellations ($N > 100$ cases).[1] The correction has no effect on the paper's results and example (i.e., Tables 1–8 in the article are identical), because all calculations with the transform were performed in the transformed space.[1] The correction also has no effect on the results of the subsequent cancellation paper,[3] because the analyses were performed without transformation. The inverse transform can readily be calculated as described by Miller.[2]

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