Opioid Self-administration in the Nerve-injured Rat

Relevance of Antiallodynic Effects to Drug Consumption and Effects of Intrathecal Analgesics

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Background: Neuropathic pain is associated with several sensory abnormalities, including allodynia as well as spontaneous pain. Opioid intake in neuropathic pain patients is motivated by alleviation of both pain and allodynia. However, laboratory animal studies rely almost exclusively on reflexive withdrawal measures of allodynia. The authors examined the pharmacology of self-regulated intake of opioids in rats with or without nerve injury and compared the rate of drug intake to reversal of allodynia.

Methods: Rats were implanted with intravenous catheters, and the L5 and L6 spinal nerves were ligated in half of these animals. Rats were then trained to self-administer a commonly abused opioid (heroin) and commonly prescribed opioids (morphine, fentanyl, hydromorphone, and methadone). In addition, rats trained to self-administer heroin were given either clonidine or adenosine spinally before self-administration sessions to assess opioid-sparing effects.

Results: Nerve injury significantly decreased the reinforcing effects of low doses of opioids, and only doses of each opioid that reduced mechanical hypersensitivity maintained self-administration after spinal nerve ligation. The rate of drug consumption was correlated with the duration of the antiallodynic effect for each dose of opioid. Intrathecal administration of clonidine or adenosine reversed mechanical hypersensitivity, but only clonidine reduced heroin self-administration in rats with spinal nerve ligation.

Conclusion: Opioid self-administration is significantly altered by nerve injury, with rate of drug intake being correlated with reversal of allodynia. Intrathecal clonidine, but not adenosine, produces opioid-sparing effects in self-administering rats. The neurobiologic mechanisms that regulate opioid consumption in rats therefore seem to be altered after nerve injury.

TREATMENT of neuropathic pain remains a poorly met medical need, and several methods of peripheral nerve injury have been studied in animals to model and further understand the pathophysiology of this disease and to develop novel treatments.1 The majority of studies use reflexive withdrawal to thermal or mechanical stimuli as measures of allodynia, and genetic and pharmacologic manipulations use this measure to gauge the relevance of specific targets to neuropathic pain. Such hypersensitivity phenomena, however, are present in far from all patients with neuropathic pain.2 Rather, ongoing pain is argued to be more important to distress and suffering in patients and the symptom for which they primarily seek treatment.2 The parallel between withdrawal responses in animals and ongoing pain in humans is uncertain, leading to a need for development of novel methods to better evaluate the pain experience after injury to guide fundamental discovery and drug development.3 This study uses drug self-administration as one such new method.

Opioids effectively reduce mechanical allodynia in animal models of chronic neuropathic pain and acutely reduce pain in humans with neuropathic pain.4–6 The need to use relatively large doses of opioids for neuropathic pain creates concern on the part of both patients and physicians over inappropriate use, addiction, and physical dependence, but whether pain alters the abuse potential of opioids has received little attention.7–9 Nerve injury diminishes the efficacy of opioids in producing conditioned place preference in rodents, an indirect method of assessing drug-seeking behavior.10,11 Drug self-administration is considered to be a more direct measure of the reinforcing effects of drugs, and this procedure assesses a number of variables relevant to drug seeking, such as the preferred rate of drug intake or factors that alter rate of drug consumption.12 When a stimulus is produced contingently on emission of a predetermined behavior, and the stimulus increases the probability of observing the contingent behavior relative to when the stimulus is not present, the stimulus is said to be reinforcing or to serve as a reinforcer.12 Drug self-administration studies assess the reinforcing effects of drugs by making drug infusions contingent on pre-determined behaviors, generally either pressing a lever or poking the nose into a receptacle with rodents.12 Drug self-administration in laboratory animals has significantly

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advanced our understanding of the neurobiology of drug abuse but has not previously been applied to determine whether chronic neuropathic pain alters this neurobiology. Adjuvant-induced arthritis alters opioid intake with a time course related to the onset and duration of hypersensitivity. Arthritic rats self-administer fewer infusions of 5 mg/kg morphine intravenously compared with normal animals, suggesting that arthritis reduces the reinforcing effects of morphine in rodents. Administration of indomethacin reduces mechanical sensitivity in arthritic rats and decreases morphine self-administration selectively in arthritic rats, suggesting that the motivation to self-administer morphine is related to pain relief in the presence of arthritis. Conversely, arthritic rats and decreases morphine self-administration selectively in arthritic rats, suggesting that the motivation to self-administer morphine is related to pain relief in the presence of arthritis. Conversely, arthritic rats self-administer morphine intravenously compared with normal animals, suggesting that arthritis decreases morphine self-administration selectively in arthritic rats, suggesting that the motivation to self-administer morphine is related to pain relief in the presence of arthritis.

The overall goal of these studies is to develop and use drug self-administration after nerve injury to probe determinants of drug use, abuse potential, and differential treatment of pain and abuse in the setting of neuropathic pain.

Materials and Methods

Subjects

Subjects consisted of 114 male, Fisher 344 rats weighing between 250 and 300 g (Charles River Laboratories, Indianapolis, IN). Animals were kept on a reversed light: dark cycle (dark 05:00-17:00 h) in a temperature- and humidity-controlled environment that was immediately adjacent to the room in which all behavioral experiments were performed. Food and water were available ad libitum to all animals used for opioid self-administration studies and acute mechanical hypersensitivity studies except during behavioral testing. Water was available ad libitum to the rats used for food reinforcement studies. All procedures are in accordance with the guidelines adopted by the Committee for Research and Ethical Issues of the International Association for the Study of Pain and were approved by the Institutional Animal Care and Use Committee of Wake Forest University, Winston-Salem, North Carolina.

Surgical Procedures

Intravenous Catheter Implantation. Animals were anesthetized with 40 mg/kg intraperitoneal pentobarbital and implanted with jugular catheters according to the methods of Weeks as previously modified. Briefly, the catheter (0.01-inch-ID Tygon tubing; Saint-Gobain Plastics, Akron, OH) was inserted into the exterior facial vein and extended through the jugular vein to just outside of the right auricle of the heart. The catheter was secured to surrounding deep muscle and to superficial neck muscle by Vicryl 4.0 suture (Ethicon Inc., Somerville, NJ) and continued subcutaneously to the back of the animal, where it exited between the scapulae through an implanted polypropylene plate encased in Teflon mesh (C.R. Bard Inc., Murray Hill, NJ). The polypropylene plate served as a point of attachment for a spring leash protecting the exterior portion of the catheter. The catheter continued through the leash, terminating at a fluid swivel. Catheter patency was maintained by hourly infusions of 0.2 ml of 0.9% saline (wt/vol) with 1.7 U/ml heparin. Rats were given 75,000 U intramuscular penicillin G procaine, and all exterior wounds were dressed with antibiotic powder (Polysporin; Pfizer Healthcare, Morris Plains, NJ).

Intrathecal Catheter Implantation. Intrathecal catheter implantation was similar to that described by Yakh and Rudy with some modifications. Catheters consisted of 8.5-cm lengths of 32-gauge polyethylene tubing (ReCathCo, Allison Park, PA) that was fused to Tygon tubing (0.01 inch ID) with cyclohexanone. The animal’s head was secured in a stereotaxic frame and an incision was made through the skin and muscle on the back of the neck. The musculature was gently retracted to expose the atlanto-occipital membrane. With the an-
imal’s head bent at approximately 80°–90° downward from the body, the catheter was inserted through a small hole made in the atlanto-occipital membrane while maintaining slight tension on the vertebral column by pulling on the tail of the rat. The catheter was secured to the surrounding muscle tissue using 5.0 Vicryl suture, and the exteriorized end of the catheter was closed using a small piece of stainless steel wire. The skin was closed using 4.0 chromic gut suture, and exterior wounds were dressed with antibiotic powder. Rats were given 10 μl sterile saline through the intrathecal catheter weekly to maintain patency.

**Spinal Nerve Ligation.** After 2–3 weeks of recovery from catheter implantation, the L5 and L6 left dorsal nerve roots were ligated as described by Kim and Chung. Animals were anesthetized with 40 mg/kg pentobarbital. Briefly, the back of the animal was shaved, and a 4-mm incision was made in the back using the iliac crest as a midpoint. An incision was then made in the underlying muscle, which was separated by both sharp and blunt dissection to expose the transverse process of the fifth lumbar vertebra. The transverse process was removed using bone microrongeurs, and the fourth and fifth lumbar nerves were exteriorized from underneath the spinal column using a small glass or metal hook. The fourth lumbar nerve was allowed to slip off of the hook, and the fifth lumbar nerve was ligated using 4.0 Vicryl silk suture with sufficient pressure to cause the nerve to bulge on each side of the ligature. The sixth lumbar nerve was exteriorized from underneath the iliac bone at the sciatic notch and ligated in a similar manner as the fifth lumbar nerve. All muscle layers and the skin were sutured using 4.0 chromic gut, and exterior wounds were dressed with antibiotic powder. Sham surgery was conducted in a similar manner, except that the L5 and L6 nerves were not ligated.

**Drugs**

Heroin hydrochloride, morphine sulfate, fentanyl citrate, and d,l-methadone hydrochloride were obtained from the Drug Supply Program of the National Institute on Drug Abuse (Rockville, MD). Heparin sodium and hydromorphone hydrochloride were purchased from Elkins-Sinn (Cherry Hill, NJ). Clonidine hydrochloride was purchased from Research Biochemicals Inc. (Natick, MA), and adenosine (Adenoscan®, Fujisawa USA, Deerfield, IL) was purchased as a 5-mg/ml sterile solution and diluted with 0.9% (wt/vol) saline. Pentobarbital was purchased from Abbott Laboratories (North Chicago, IL), and penicillin G procaine was purchased from Butler Vet (Columbus, OH). The doses of all drugs are given in terms of the free base weight, and all drug solutions were sterilized by filtration through a 0.22-μm nitrocellulose filter.

**Drug Administration**

**Intravenous Drug Administration.** All drugs administered intravenously were given in a volume of 1 ml/kg body weight in sterile 0.9% NaCl (wt/vol) with 1.7 U/ml heparin. For determination of potency and efficacy of reversal of mechanical hypersensitivity, each dose was introduced into the catheter and flushed with 0.5 ml sterile 0.9% NaCl with heparin.

**Intrathecal Drug Administration.** All drugs were given intrathecally in a volume of 10 μl, and the catheter was flushed with 12 μl sterile 0.9% NaCl. Infusions were administered using a microsyringe infusion pump (KD Scientific, Boston, MA) and a 25-μl Hamilton microsyringe at a flow rate of 22 μl/min.

**Behavioral Procedures**

**Determination of Paw Withdrawal Threshold.** Paw withdrawal threshold was determined according to previously published methods using von Frey filaments ranging in strength from 0.4 to 15.0 g. For determination of the antiallodynic effects of methadone and hydromorphone, paw withdrawal threshold values were calculated before and 1, 3, 5, 10, 20, 30, 45, and 60 min after drug treatment using Dixon nonparametric statistics. The experimenter was not blinded to drug or dose. Sedation after drug administration was defined as reluctance to move in the chamber and ataxia as the inability of the animal to right itself on all four paws. After 5–7 days of recovery from nerve ligation surgery, the withdrawal threshold was determined, and animals were considered to be alldynic if the withdrawal threshold was 4.0 g or less. Alldynia was verified in spinal nerve-ligated (SNL) rats using von Frey filaments at least twice per week for animals used in opioid and food self-administration studies to ensure that mechanical hypersensitivity remained throughout the duration of the experiment.

**Opioid Self-administration.**

**Apparatus.** Commercially available operant equipment was used consisting of an operant chamber containing a lever located 5 cm above a grid bar floor, a stimulus lamp located 2 cm above the lever, a house light located outside of the operant chamber, a pellet receptacle, a magazine-type pellet dispenser, and a tone generator (Med Associates Inc., St. Albans, VT). Each operant chamber was placed within a sound- and light-attenuating enclosure containing a ventilation fan. An infusion pump was located on the outside of the chamber.

**Procedure.** A within-session dose–response procedure was used to engender and maintain responding by opioid infusions as described previously. One of four doses of each drug was made available for a 1-h component during each self-administration session, and each hourly component was separated by a 20-min period during which lever presses had no programmed consequences. The doses were altered by varying the time of...
operation of the infusion pump. The order of dose availability was descending, such that the highest dose of drug available for each session was presented in the first hourly component followed by sequentially lower doses in each of the remaining hourly components. The dose order was reversed to an ascending series in one group of SNL and sham rats trained to self-administer heroin. For the training of acquisition of self-administration, each lever press resulted in delivery of an infusion of the dose and opioid available for that hourly component of the self-administration session (fixed ratio 1 schedule). Each rat was exposed to only one opioid, and the opioid studied for each rat was used for both acquisition of self-administration and for analysis of stable intake after acquisition. Stable intake was defined as five successive self-administration sessions during which the number of infusions per hour for each dose of opioid studied did not vary by more than 15% of the mean value obtained across those 5 sessions. Once stable intake was obtained at fixed ratio 1, the number of lever presses required for an infusion was gradually increased to a terminal value of 10 (fixed ratio 10) across several experimental sessions. Illumination of the stimulus light above the lever indicated drug availability and a time-out period of 30 s followed the delivery of each infusion during which a tone was activated, the stimulus light above the lever was turned off, and lever presses had no programmed consequences. Sessions were conducted during the dark phase of the light:dark cycle on weekdays only. The total number of drug infusions administered was recorded for each dose as well as the time elapsed between each individual infusion, and no infusion limits were imposed for any of the animals or for any of the opioids studied.

**Food Reinforcement.**

*Apparatus.* The apparatus used for these studies was similar to that described above for the drug self-administration experiments. In addition, water was provided *ad libitum* by a water bottle located on one wall of the operant chamber.

*Procedure.* Lever presses were engendered and maintained by presentation of standard 45-mg rat chow pellets (Research Diets Inc., New Brunswick, NJ). Initially, each lever press resulted in delivery of a food pellet, and the number of lever presses required to earn a pellet was gradually increased to a terminal value of 10 across several experimental sessions. Illumination of the stimulus light above the lever indicated pellet availability, and a time-out period of 5 s followed the delivery of each pellet during which the tone was activated, the stimulus light above the lever was turned off, and lever presses had no programmed consequences. Animals were required to earn all of their daily food ration by pressing the lever and food was available 24 h/day. Animals were kept on the same reversed light:dark cycle as the other animals used in this study. The total amount of food pellets earned and the amount of food pellets earned within each individual hour of the day were recorded using commercially available software (Med-PC; Med Associates).

**Data and Statistical Analyses**

Data for paw withdrawal threshold measures were analyzed using a two-way analysis of variance with drug dose and time after infusion serving as the independent variables. The half-life of the effect of each dose on paw withdrawal threshold was calculated assuming monoexponential kinetics using Prism software (Graph Pad, San Diego, CA). The data for opioid self-administration was analyzed using a two-way analysis of variance with dose and treatment condition (nerve ligation or sham surgery) as the independent variables and number of infusions serving as the dependent measure. The data for opioid self-administration following intrathecal clonidine or adenosine was analyzed using two-way analysis of variance with intrathecal clonidine and intravenous heroin dose as the two independent variables and number of infusions as the dependent measure in groups of rats after either nerve ligation or sham surgery. *Post hoc* analyses were made using the Tukey least significant differences *t* test with commercially available software and a Macintosh computer (JMP; SAS Institute, Research Triangle Park, NC).

**Results**

*Reversal of Mechanical Alloodynia by Intravenous Opioids* The effects of intravenous administration of heroin, morphine, and fentanyl on mechanical allodynia after L5–L6 SNL have been reported previously. Methadone produced a dose-dependent reversal of mechanical allodynia after SNL in the dose range of 100–600 μg/kg, with 600 μg/kg producing a maximal effect (15 g) (fig. 1A). The peak effect for all doses occurred at the earliest time point tested (1 min). The largest dose tested (1 mg/kg) produced a maximal effect accompanied with significant sedation and ataxia present in the first 20 min, making accurate withdrawal thresholds difficult to determine. Paw withdrawal threshold returned to baseline levels 10, 30, or 60 min after administration of 100, 300, or 600 μg/kg methadone, respectively. Hydromorphone produced a dose-dependent reversal of mechanical allodynia after intravenous administration of 10–30 μg/kg, producing a maximal threshold of 11.5 (2.5) g (fig. 1B). The effects were time dependent, with paw withdrawal threshold returning to baseline values at 20 or 30 min after administration of 10 or 30 μg/kg, respectively. Administration of 100 μg/kg hydromorphone produced significant sedation and ataxia similar to that found with the highest dose of methadone for the first 20 min after intravenous administration and accurate paw withdrawal thresholds were not achieved until 50 min after injection.
Effects of SNL on Opioid Self-administration

**Heroin.** Spinal nerve ligation produced significant effects on the rate of self-administration of heroin compared with sham rats, and the effect was dependent on the amount of heroin administered per injection and the order of dose presentation (figs. 2 and 3). The average paw withdrawal threshold for SNL rats trained to self-administer heroin was 1.8 (0.5) g compared with 14.5 (1.2) g for sham rats. The ability of infusions of heroin to reinforce lever pressing increased as the doses were increased up to 15 μg/kg, and then decreased at higher doses presumably due to pharmacokinetic factors. Rate of lever pressing varied across the range of doses studied in both SNL (F7,47 = 42.2, P < 0.0001) and sham (F7,47 = 12.5, P < 0.0001) animals, and there was a significant interaction between heroin dose and ligation or sham surgery (F7,95 = 14.4, P < 0.0001). All doses of heroin greater than 4.5 μg/kg maintained self-administration in sham-treated subjects; however, post hoc comparisons found that the rate of self-administration of 9 and 15 μg/kg heroin was significantly less in SNL compared with sham-ligated animals (fig. 2). There was no significant difference in the maximum rate of lever pressing that could be obtained with heroin between SNL and sham-treated animals (P ≤ 0.05). Only doses of 18 μg/kg heroin or higher maintained self-administration at a rate greater than that maintained by saline after SNL. These data were collected by making the highest dose of heroin available for self-administration first, followed by sequential decreases in the heroin dose across the hourly components of the session. When the order of dose presentation was reversed, such that the lowest dose of heroin occurred first within the sequence of 18, 30, 60, or 100 μg/kg, even greater differences in the rate of drug intake were observed (fig. 3). There was a significant effect of dose order between SNL and sham rats (F1,47 = 75.2, P < 0.0001) and a significant interaction between dose order and heroin dose in SNL animals (F3,47 = 19.5, P < 0.0001). Heroin intake was significantly lower for 18 and 30 μg/kg when these doses occurred first and second, respectively, within the session compared with when self-administration was initiated by the higher dose occurring earlier in the session (P < 0.05). No such

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**Fig. 1.** Reversal of mechanical hypersensitivity by methadone and hydromorphone. Methadone (A) or hydromorphone (B) was administered intravenously 20 min after determination of baseline paw withdrawal threshold, and withdrawal thresholds were determined at the indicated times after infusion. Data indicate mean (SEM), n = 6 for each compound.

**Fig. 2.** Opioid self-administration after spinal nerve ligation or sham treatment. The dose effect curves for the self-administration of each opioid were determined across several self-administration sessions as described in the Materials and Methods. The x-axis indicates the dose of opioid per self-administered infusion, and the y-axis represents the number of infusions self-administered per hour. Mechanical allodynia was verified in spinal nerve-ligated rats using von Frey filaments and defined as a paw withdrawal threshold of 4 g or less. n = 6 rats/group with separate groups studied for each drug. * Significantly different from sham, P < 0.05.
differences were found for sham-treated rats when the order of the available doses was reversed (fig. 3).

**Morphine.** The average paw withdrawal threshold for the SNL group used for morphine self-administration was 2.5 (0.4) g, and that for the sham group was 14.8 (0.4) g. As with heroin, morphine maintained self-administration in sham subjects with a typical inverted U-shaped function that was dose dependent (F = 8.6, P < 0.0001) (fig. 2). In SNL animals however, morphine self-administration was not dose dependent (F = 2.1, P = 0.07), and there was a significant difference between the dose-effect curves for morphine self-administration in SNL compared with sham-treated subjects (F = 3.6, P = 0.002). The maximum response rate (lever presses/h) that could be obtained with morphine was significantly less in SNL rats compared with sham-treated animals (P ≤ 0.05). *Post hoc* comparisons found significant differences between the rate of self-administration of doses of morphine lower than 180 µg/kg per infusion that maintained robust self-administration in sham-treated but not SNL animals (fig. 2).

**Fentanyl.** The effect of SNL on fentanyl self-administration was similar to the effect produced on behavior maintained by infusions of morphine (fig. 2). The average paw withdrawal threshold for the SNL group was 2.2 (0.3) g, and that for the sham group was 14.8 (0.5) g. Fentanyl maintained self-administration in a dose-dependent manner in sham (F = 4.1, P = 0.002) but not SNL rats (F = 1.6, P = 0.13), and the dose-effect curves were significantly different between these groups (F = 2.2, P = 0.04) (fig. 2). As with morphine, the maximum rate of lever pressing that could be obtained with fentanyl infusions was significantly less with SNL rats than that found in sham-treated subjects (P ≤ 0.05).

**Methadone.** Similar to heroin, methadone maintained self-administration in a dose-dependent manner in both sham (F = 2.55, P = 0.05) and nerve-injured (F = 2.48, P = 0.03) subjects (fig. 2). The average paw withdrawal threshold for the SNL group was 2.1 (0.3) g, and for the sham group was 15 (0) g. The dose-effect curves were significantly different between these groups (F = 2.33, P = 0.04); however, the maximum rate of responding that could be obtained with methadone was not different between SNL and sham-treated subjects. Doses of 38 and 75 µg/kg methadone did not maintain self-administration in SNL animals but did so in sham-treated subjects (P ≤ 0.05), consistent with the dose range of methadone that produced a reversal of mechanical hypersensitivity (fig. 1).

**Hydromorphone.** Hydromorphone maintained self-administration in a dose-dependent manner in sham-treated (F = 6.8, P < 0.0001) and SNL (F = 3.8, P = 0.004) animals (fig. 2). The average paw withdrawal threshold for the SNL group was 1.9 (0.6) g, and that for the sham group was 14.6 (0.8) g. There was a significant difference in the dose-response curves between these two groups (F = 4.27, P = 0.04) and doses of 2.7, 4.5, 15, and 18 µg/kg hydromorphone maintained self-administration at much lower rates after SNL compared with sham treatment (P ≤ 0.05). The maximum in the self-administration dose-effect curve was decreased for hydromorphone in nerve-injured compared with sham-treated subjects (P ≤ 0.05), similar to morphine and fentanyl.

**Comparison of Rate of Drug Consumption with Duration of Antiallodynic Effects of Intravenous Opioid Administration**

The rate of drug intake was compared with the calculated duration for reversal of hypersensitivity for the doses of opioids that maintained self-administration in SNL rats and for which time course studies were conducted in the current study or from antiallodynia data obtained previously (table 1). A positive correlation was found between the interreinforcement interval (the time between each infusion) for each dose of heroin that maintained responding in SNL animals and the half-life for reversal of mechanical hypersensitivity (r² = 0.74). The slope of the least-squares fitted line was not significantly different from 1.0 (0.901 [0.15]). For each dose of opioid that maintained self-administration, the predicted paw withdrawal threshold for the amount of time elapsed between self-administration of infusions was below 4.0 g, with the exception of the highest dose of heroin studied (table 1).

**Food-maintained Responding in SNL and Sham-treated Subjects**

In contrast to the behavior maintained by opioids, food reinforced behavior was not affected by either nerve...
ligation or sham treatment compared with untreated rats that were removed from the operant chamber for 7 days (fig. 4). Basal rate of responding for food before surgery was not significantly different between the groups used for SNL (372 ± 7.5 pellets/day) or sham surgery (374 ± 8.7 pellets/day) or groups that were not treated (376 ± 7.1 pellets/day) (F2,35 = 0.05, P = 0.95). The peak hourly response rate also did not differ between these groups before surgery (F2,17 = 0.72, P = 0.5) (SNL: 64 ± 2.1 pellets/h; sham: 68 ± 2.1 pellets/h; naive: 65 ± 2.6 pellets/h). There was no effect of nerve ligation, sham surgery, or simply removing the animal from the operant chamber for 7 days (naive) on the total number of pellets earned throughout 24 h compared with basal values (F2,35 = 0.5, P = 0.61) (SNL: 378 ± 11.8 pellets/day; sham: 396 ± 12.6 pellets/day; naive: 372 ± 19.1 pellets/day) and no interaction between surgical treatment and treatment group (F2,35 = 0.6, P = 0.55). Similarly, SNL or sham surgical treatment had no effect on the peak hourly response rate recorded over a 24-h period in these groups of rats (F2,35 = 0.78, P = 0.47) (SNL: 66 ± 3.9 pellets/h; sham: 70 ± 5.5 pellets/h; naive: 69 ± 3.0 pellets/h).

Effects of Intrathecal Analgesics on Mechanical Hypersensitivity and Heroin Self-administration in Nerve-injured Rats

Clonidine. Intrathecal administration of 10 µg clonidine reversed mechanical allodynia beginning 10 min after administration, increasing the paw withdrawal threshold from 1.8 (0.2) to 13.2 (0.4) g (F5,65 = 55.6, P < 0.0001). Mechanical thresholds gradually returned to baseline levels over 2.5 h. Administration of 10 µg clonidine intrathecally decreased heroin intake in SNL rats, and there was a significant interaction between the effect of clonidine and the dose of heroin being self-administered (F6,98 = 5.4, P < 0.0001) (fig. 4). Administration of 10 µg intrathecal clonidine had no effect on heroin self-administration in sham-treated subjects (F6,98 = 0.8, P = 0.54) (fig. 4). Administration of 30 µg intrathecal clonidine produced significant sedation that was observed in all animals with the rats lying prostrate on the operant chamber floor, and decreased heroin intake in both SNL and sham-treated subjects to a similar extent (data not shown). Administration of saline intrathecally was without effect in either group (P > 0.05) (fig. 4).

Adenosine. Intrathecal administration of 30 µg adenosine reversed mechanical allodynia in SNL rats beginning 1 h after administration, increasing the paw withdrawal threshold from 2.3 (0.4) to 11.4 (2.2) g, and the effect lasted for up to 7 h (F6,65 = 63.9, P = 0.0001). Despite this effect on mechanical hypersensitivity, intrathecal administration of 30 µg adenosine did not significantly alter the rate of heroin consumption in either nerve-injured or sham-injured animals (fig. 5). There was

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no significant main effect of adenosine administration (F_{1.35} = 1.6, P = 0.22) and no interaction between adenosine treatment and heroin dose (F_{2.35} = 1.5, P = 0.24) in SNL rats. There was also no effect of adenosine treatment on heroin intake in sham-treated rats (F_{1.35} = 1.8, P = 0.19) and no interaction between adenosine treatment and heroin dose (F_{2.35} = 1.8, P = 0.18).

**Discussion**

There is a clear need for development of measures of pain and hypersensitivity in animals that address more than spinally mediated reflexes. The current study establishes that there is a differential pharmacology for opioids in maintaining self-administration after SNL compared with sham treatment in rats, in terms of the opioids that maintain self-administration, the dose–response curves for self-administration, and the effects of intrathecal clonidine on heroin intake. Each opioid maintained self-administration in nerve-injured animals only at doses that produced a reversal of mechanical hypersensitivity and at a rate of consumption that was consistent with the duration of reversal of tactile allodynia. Only heroin and methadone produced a maximal effect against the mechanical stimulus without producing sedation and ataxia. These two opioids were also the only compounds for which the maximal response rate was not suppressed for self-administration after nerve injury. The rate of drug intake of all doses of opioids that maintained self-administration was consistent with their time course for producing a reversal in mechanical hypersensitivity as measured using paw withdrawal threshold with the exception of the highest dose of heroin (100 μg/kg). These data support the notion that the reinforcing effects of opioids are diminished in nerve-injured subjects relative to sham animals, particularly at doses of opioids that fail to reverse hind-paw hypersensitivity after SNL surgery.

The current data also pertain to the ongoing discussion and controversy of the addiction liability of opioid drugs when administered or self-administered within a chronic pain setting. A valid interpretation of this data is that the addiction liability of heroin and methadone is significant at higher doses in the presence of untreated neuropathic pain, similar to the addiction potential in normal subjects. Alleviation of neuropathic pain with intrathecal clonidine effectively reduces the addiction liability of heroin in animals that have experienced the subjective effects of heroin only after nerve injury, but not in subjects that have experienced the subjective effects of heroin in the absence of nerve injury. These data therefore predict that undertreatment or failure to treat neuropathic pain increases the propensity for opioid-seeking behavior.

Interpretation of the inverted U-shaped dose–effect function that is obtained using fixed-ratio schedules of drug self-administration is controversial. One interpretation is that the rate of operant responding increases with increasing amount of drug per infusion at the lower end of the dose effect due to an increase in the production of the neurochemical effects or neuronal activities that comprise the reinforcing cue for that particular drug. The descending limb, in which the rate of drug intake or lever pressing decreases as the amount of drug administered per injection increases, is thought to occur due to pharmacokinetic factors, production of behaviors that interfere with operant responding such as sedation or catalepsy, or production of aversive subjective cues that compete with positive reinforcing cues. Given this interpretation of the self-administration dose–effect curves in the current study, SNL surgery diminishes the positive reinforcing effects of opioids, because doses that result in increasing rates of behavior with increasing dose per injection in sham rats maintain significantly lower rates of operant responding in SNL rats. At higher doses, the rate of drug intake is consistent with reversal of hypersensitivity in SNL rats. A similar downward shift in the dose–effect curve for self-administration of low doses of heroin is produced by perinatal lead exposure in rats, a manipulation found to diminish positive reinforcement by opioids.
The food reinforcement data suggest that the effect of peripheral nerve injury on opioid reinforcement is not due to a generalized physical debilitation that prevents the rat from pressing the lever or maintaining sufficient activity to obtain drug infusions. The lever height (5 cm) above the grid bar floor necessitates that the animal rear on its hind limbs to press the lever and therefore place load-bearing weight on the injured hind paw for both food and opioid reinforcement. This suggests that nerve injury selectively attenuates the positive reinforcing effects of opioids rather than operant behavior in general and is in agreement with findings that peripheral nerve injury or chronic hind-paw inflammation attenuates opioid reinforcement using conditioned place preference.\textsuperscript{10,11} Conditioned place preference has proven to be a valuable tool for examining reinforcement mechanisms but, unlike self-administration, relies on experimenter-delivered injections and cannot address mechanisms of self-regulated drug consumption. The current data suggest that the self-administration model will be valuable to develop the pharmacology and neurobiology of brain and spinal mechanisms that pertain specifically to opioid consumption with this neuropathic pain model. In addition, the current animal model is easily adapted to the study of dose escalation and tolerance after chronic treatment by permitting continuous access to opioids by self-administration.

One possible interpretation of the effects of SNL on opioid self-administration might be the presence of chronic stress or anxiety in the allodynic rats. Chronic pain in humans is associated with comorbidity of several indices of psychological dysfunction, including depression, anxiety, and chronic stress.\textsuperscript{34} The effect of stress on drug reinforcement has been assessed with a number of laboratory models, and the predominant effect is an increase in drug intake. The most consistent finding is that acute application of stressful stimuli will increase opioid-seeking behaviors in rats, an effect that is mediated by noradrenergic systems and corticotrophin-releasing factor.\textsuperscript{35,36} Involvement of the hypothalamic–pituitary axis in this stress response is variable and seems to be stressor, drug, and context specific.\textsuperscript{35} It is noteworthy that application of a mild electric foot shock has no effect on the self-administration of infusions of 100 $\mu$g/kg heroin under a fixed-ratio schedule in agreement with the current findings, but increases self-administration under a progressive ratio schedule of reinforcement under which the ratio requirement for delivery of infusions increases incrementally with subsequent infusions.\textsuperscript{37} Therefore, other schedules of reinforcement may be able to delineate effects of SNL on drug reinforcement that fixed-ratio schedules do not elucidate. Interestingly, intracerebroventricular administration of clonidine prevents stress-induced heroin-seeking behaviors in rats, similarly to the findings in the current study with intrathecal administration of clonidine in SNL rats.\textsuperscript{38} This may indicate that clonidine produces pertinent supraspinal effects after intrathecal administration relevant to opioid consumption. Others have found that the hypothalamic–pituitary axis is not chronically activated in neuropathic pain models in rats.\textsuperscript{39,40} Rather, the presence of neuropathic pain seems to increase corticotrophin-releasing factor levels in amygdala, as well as produce diminished activation of second-messenger systems by opioids in this structure and produce anxiogenic effects in mice.\textsuperscript{40,41} The amygdala is rich in both $\alpha_2$- and $\alpha_2c$-adrenergic receptors and could potentially serve as a supraspinal site of action for the effects of intrathecal clonidine found in the current study.\textsuperscript{42,43} The effects of SNL on opioid self-administration may therefore be related to anxiogenesis, rather than the presence of acute stress.

Methadone and heroin were relatively more effective than morphine in reversing the effects of nerve injury on mechanical hypersensitivity and maintaining self-administration in nerve-injured rats. Regarding methadone, there are reports that this drug produces greater analgesia in patients with neuropathic pain after failure of morphine therapy.\textsuperscript{44} Possible explanations for an enhanced effect of methadone over morphine could include a higher relative intrinsic efficacy at $\mu$-opioid receptors or possibly antagonism of $N$-methyl-$\textit{D}$-aspartate receptors by d-methadone.\textsuperscript{45,46} $N$-Methyl-$\textit{D}$-aspartate antagonists possess antiallodynic effects in rats after SNL and potentiate the analgesic effects of opioids in rats after SNL.\textsuperscript{47,48} Clearly, there are no clinical studies examining heroin use for chronic pain in the clinic; however, a major metabolite of heroin, 6-monoacetylmorphine, also displays higher relative intrinsic efficacy at $\mu$-opioid receptors compared with morphine.\textsuperscript{39} Pharmacokinetic considerations may be responsible as well, because higher peak concentrations of total opioid can be achieved in brain after intravenous administration of heroin compared with morphine, and at a faster rate.\textsuperscript{50}

The differential effects of intrathecal administration of clonidine and adenosine on heroin consumption in SNL rats suggest that although mechanical hypersensitivity seems to have some role in the behavioral effects of nerve injury, other variables may contribute to the modulation of opioid consumption as well in this rat model of neuropathic pain. We speculate that the selective decrease in heroin intake by intrathecal administration of clonidine but not adenosine in SNL rats supports the concept that opioid self-administration in this model is related to ongoing or spontaneous pain. Supporting evidence comes from clinical data indicating that intrathecal administration of both adenosine and clonidine alleviate elicited pain (allodynia) in patients with neuropathies, but only clonidine is effective against spontaneous (ongoing) pain.\textsuperscript{19,20} Opioids alleviate ongoing pain in patients with peripheral nerve injury.\textsuperscript{51} Others have suggested that L5–L6 nerve ligation produces...
spontaneous pain due to behavioral observations, such as altered posture of the animal and ventroflexion of the affected hind paw.52 Such measures can be difficult to standardize and quantify however. Hind-limb weight-bearing behavior is altered after SNL in rats such that a greater percentage of weight is placed on the hind paw contralateral to nerve injury.52 Because this assay measures behavior in the absence of an external stimulus, this behavior is thought to be indicative of ongoing or spontaneous pain. Milnacipran, a norepinephrine/serotonin uptake inhibitor, produces a greater reversal of the effects of SNL on weight-bearing behavior compared with mechanical hypersensitivity after both acute and chronic administration in rats.52 Similar to the current data, this suggests that the mechanisms of spontaneous and elicited pain may differ after nerve injury.

In conclusion, the current data indicate that the pharmacology of opioids in maintaining self-administration differs between sham rats and those after SNL. Higher doses of opioids are required to maintain self-administration in rats after SNL compared with sham animals, and the time between self-administered infusions of each dose of opioid is related to the duration of the reversal of mechanical hypersensitivity of the hind paw using traditional von Frey filament testing. The differences between the effects of intrathecal clonidine administration on heroin self-administration in SNL compared with sham rats further suggests that spinal analgesic mechanisms may be relevant to opioid intake selectively in the nerve-injured rats. Hopefully, the continued study of the basic mechanisms that are responsible for the differential regulation of opioid intake between nerve-injured and sham animals will lead to improved therapy and opioid use with neuropathic pain with diminished addiction liability.

References

3. Mogil JS, Crager SE: What should we be measuring in behavioral studies of chronic pain in animals? Pain 2004; 112:12–5
Nanjo K, Matsuzawa K, Yamasaki M, Suzuki T: Chronic pain induces anxiety with concomitant changes in opioidergic function in the amygdala. Neuropsychopharmacology 2006; 31:739–50