Involvement of the Lateral Amygdala in the Antiallodynic and Reinforcing Effects of Heroin in Rats after Peripheral Nerve Injury

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ABSTRACT

Background: Neuropathic pain alters opioid self-administration in rats. The brain regions altered in the presence of neuropathic pain mediating these differences have not been identified, but likely involve ascending pain pathways interacting with the limbic system. The amygdala is a brain region that integrates noxious stimulation with limbic activity.

Methods: μ-Opioid receptors were blocked in the amygdala using the irreversible antagonist, β-funaltrexamine, and the antiallodynic and reinforcing effects of heroin were determined in spinal nerve-ligated rats. In addition, the effect of β-funaltrexamine was determined on heroin self-administration in sham-operated rats.

Results: β-Funaltrexamine decreased functional activity of μ-opioid receptors by 60 ± 5% (mean ± SD). Irreversible inhibition of μ-opioid receptors in the amygdala significantly attenuated the ability of doses of heroin up to 100 μg/kg to reverse hypersensitivity after spinal nerve ligation. Heroin intake by self-administration in spinal nerve-ligated rats was increased from 5.0 ± 0.3 to 9.9 ± 2.1 infusions/h after administration of 2.5 nmol of β-funaltrexamine in the lateral amygdala, while having no effect in sham-operated animals (5.8 ± 1.6 before, 6.7 ± 0.9 after). The antiallodynic effects of 60 μg/kg heroin were decreased up to 4 days, but self-administration was affected for up to 14 days.

Conclusions: μ-Opioid receptors in the lateral amygdala partially meditate heroin's antiallodynic effects and self-administration after peripheral nerve injury. The lack of effect of β-funaltrexamine on heroin self-administration in sham-operated subjects suggests that opioids maintain self-administration through a distinct mechanism in the presence of pain.

What We Already Know about This Topic

- Opioid misuse in chronic pain patients is a serious concern
- In animal models of persistent pain, the pharmacology of opioid self-administration, a correlate of drug misuse, is different from that of normal animals

What This Article Tells Us That Is New

- The lateral amygdala is a key area for opioid self-administration in a persistent neuropathic pain model and may be a target for preventing drug misuse in persistent pain

SEVERAL studies have suggested that opioid consumption through self-administration is altered by the presence of pain in laboratory animals. In rats with experimental arthritis, morphine self-administration is decreased after alleviation of inflammation by indomethacin treatment, suggesting that morphine intake is at least partially dependent upon the extent of pain.[1] Oral fentanyl self-administration is approximately seven times greater in arthritic rats compared with control animals, and intake increases with a time course comparable to the progression of inflammation.[2,3] In addition, oral fentanyl self-administration is selectively decreased during chronic dexamethasone treatment only in arthritic rats.[3] Intracerebroventricular self-administration of morphine increases when painful electrical shock is delivered in rats, suggesting that rats are capable of titrating analgesia by self-administration.[4] We have previously shown that tight ligation of the L5 and L6 nerves, a model of neuropathic pain, significantly modifies the pharmacology of opioid self-administration in rats.[5] In that study, morphine and fentanyl had weak activity in reversing mechanical hypersensitivity in spinal nerve-ligated (SNL) rats and likewise did not maintain

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robust self-administration in these animals. Heroin and methadone were more efficacious both in reversing mechanical hypersensitivity and maintaining self-administration, and the rate of intake through self-administration was consistent with maintenance of antiallodynic effects in SNL rats. Clonidine administration (10 μg, intrathecal) reversed mechanical hypersensitivity in SNL rats and selectively decreased heroin self-administration in these animals, while having no effect on heroin intake in sham-operated controls. The conclusion from these experiments was that L5/L6 ligation produces a distinct pharmacology of opioids as measured using drug self-administration, likely mediated through brain regions that are influenced by spinal input. Identifying these regions and the relevant neurons may provide a strategy for improving chronic pain treatment with reduced abuse liability.

The brain contains many regions rich in μ-opioid receptors (MORs), including regions involved in both drug reinforcement (*e.g.*, nucleus accumbens, ventral tegmental area, and prefrontal cortex) and analgesia (*e.g.*, periaqueductal gray, anterior cingulate cortex, and dorsal raphe).[6] MORs are also densely located in the amygdala, a region that is involved in both analgesia and drug-reinforcement mechanisms.[6] Injection of opioids into the amygdala produces analgesia in rodents through MORs.[7] MOR activation in the amygdala ipsilateral to application of a persistent painful stimulus in the masseter muscle in humans is associated with a decrease in verbal pain scores, a measure of the affective response to pain.[8] μ-Opioids appear to induce analgesia in the amygdala by inhibiting GABAergic projection neurons from the basolateral amygdala (BLA) to the central nucleus of the amygdala (CeA), with the CeA sending projection neurons to the periaqueductal gray (PAG), which, in turn, projects to the rostroventral medulla (RVM).[9] The BLA also contains excitatory glutamatergic neurons that project to the nucleus accumbens and medial prefrontal cortex, and these projections are thought to synapse directly on dopaminergic nerve endings.[10,11] An amygdalonigral pathway has been described using anterograde labeling to identify projections from the CeA to the substantia nigra and ventral tegmental area.[12] Stimulation of inhibitory GABAergic fibers in the CeA that project to the ventral tegmental area modulate dopaminergic projection neurons from the ventral tegmental area that are involved in reinforcement circuitry.[13,14] Therefore, the amygdala modulates regions involved in both sensory processing and limbic activity.

Given its ability to modulate pathways involved in either pain or drug reinforcement, the amygdala is a brain region that could possibly be responsible for the differential pharmacology of opioids in SNL and sham-operated rats reported previously by our laboratory. To determine whether MORs in the BLA were involved in either the antiallodynic or reinforcing effects of heroin in SNL rats, the irreversible MOR antagonist, β-funaltrexamine (β-FNA), was administered directly into this region. The behavioral effects of heroin were then determined and compared with those obtained from sham-operated rats. The anatomical distribution and extent of β-FNA's effect on MOR activity was determined in a separate group of rats administered 2.5 nmol of β-FNA or saline into the lateral amygdala by examining stimulation of [^{35}S]guanosine 5′-*O*-(3-thiotriphosphate) ([^{35}S]GTPγS) binding by the MOR agonist, [D-Ala^2,NMe-Phe^4, Gly-ol^5]enkephalin (DAMGO).

Materials and Methods

Subjects

Subjects consisted of 95 male Fisher 344 rats weighing between 250 and 300 g (Harlan Sprague-Dawley, Indianapolis, IN). Animals were kept on a reversed light-dark cycle (dark 5:00 AM–5:00 PM) and given *ad libitum* access to food and water, except during self-administration sessions or during testing with von Frey filaments. All procedures were approved by the Animal Care and Use Committee of Wake Forest University Health Sciences (Winston-Salem, NC) and were in accord with the Guidelines for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (Bethesda, MD).

Surgical Procedures

Intravenous Catheter Implantation. Animals were anesthetized with an intraperitoneal injection of 40 mg/kg pentobarbital and implanted with jugular catheters, according to the methods of Weeks, as previously modified.[15,16] In brief, the catheter (0.01-in ID Tygon tubing, type S-54-HL; Saint-Gobain PPL Corp., New Haven, CT) was inserted into the right 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 and continued subcutaneously to the back of the animal, where it exited between the scapulae through an implanted Teflon plate (Small Parts, Inc., Miami Lakes, FL) encased in Teflon mesh (Davol Inc., Warwick, RI). The Teflon 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.[17] Catheter patency was maintained by hourly infusions of 0.2 ml 0.9% saline (w/v) with 1.7 U/ml heparin. Rats were given 75,000 U of penicillin G procaine intramuscularly, and all exterior wounds were dressed with antibiotic powder (Polysporin; Johnson and Johnson, Skillman, NJ).

Intracranial Guide Cannula Implantation. Rats were implanted with 22-gauge stainless steel injection guide cannulas (Plastics One, Roanoke, VA) aimed at the lateral portion of the amygdala (5.6 mm rostral from λ, ± 4.5 mm lateral from the midline, and 6.8 mm ventral from the skull surface) using a stereotaxic frame (Stoelting, Wood Dale, IL). The guide cannulas were secured to the skull with stainless-steel screws and dental acrylic cement. Dummy cannulas (28 g) were

inserted to prevent blockage. All animals were implanted with a bilateral guide cannula, with the exception of those used for DAMGO-stimulated [^{35}S]GTPγS binding in brain sections, as noted below.

Spinal Nerve Ligation. After 2 weeks of recovery from catheter and guide cannula implantation, the L5 and L6 left dorsal nerve roots were ligated as described by Kim and Chung (1992) under isoflurane anesthesia (induction with 5% saturation, maintenance with 2–3% saturation).[18] A deep midline incision of 4 cm was made in the back of the animal using the iliac crests as the midpoint. After removal of the fifth transverse process with microrongeurs, L5 was exteriorized and ligated with a 4.0 silk suture. L6 was exteriorized from underneath the iliac bone and ligated in a similar manner. The muscle was closed with 4.0 chromic gut interrupted sutures, and the skin was closed separately in a similar manner. Sham ligation surgery was the same, except that the L5 and L6 nerves were not ligated.

Determination of the Extent of Irreversible Inhibition of MOR by β-FNA in the Amygdala Using DAMGO-stimulated [^{35}S]GTPγS Binding in Brain Sections

Rats were implanted with a single guide cannula into the left lateral portion of the amygdala as described above and administered either 1.0 μl saline (n = 8) or 2.5 nmol of β-FNA (n = 8) as described above. Rats were then sacrificed 24 h later, and their brains were removed and frozen in isopentane (−35°C) and stored at −80°C until assayed. Coronal sections (20 μm) were obtained in a cryostat at −20°C and assayed for [^{35}S]GTPγS binding as described previously.[19] Sections were rinsed in assay buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 0.2 mM EGTA, 100 mM NaCl, pH 7.4) at 25°C for 10 min, followed by a 15-min preincubation in assay buffer containing 2 mM guanosine diphosphate and 10 mU/ml adenosine deaminase at 25°C. Sections were then incubated in assay buffer with 2 mM guanosine diphosphate, 10 mU/ml adenosine deaminase, and 0.04 nM [^{35}S]GTPγS, with (stimulated) or without (basal) 10 μM DAMGO at 25°C for 2 h. Slides were rinsed twice in cold Tris buffer (50 mM Tris-HCl, pH 7.0) for 2 min and once in deionized water for 30 s.[20] After drying at room temperature overnight, sections were exposed to film for 72–96 h in cassettes containing [^{14}C] standards for densitometric analysis. Films were digitized with a Sony XC-77 video camera (Sony Corp., Tokyo, Japan) and analyzed densitometrically using the National Institutes of Health Image program for Macintosh computers version 1.6 (National Institutes of Health, Rockville, MD). Values were corrected for [^{35}S] based on incorporation of [^{35}S] into sections of frozen brain paste as previously described, and correction factors were used to convert [^{14}C] values to [^{35}S] data. Net agonist-stimulated [^{35}S]GTPγS binding was calculated by subtracting basal binding from agonist-stimulated binding in triplicate sections for each animal. Data are expressed as nCi [^{35}S]GTPγS/g tissue and are reported as mean values ± SD.

Drugs and Reagents

Heroin hydrochloride was obtained from the Drug Supply Program of the National Institute on Drug Abuse (Rockville, MD). Solutions were prepared in sterile 0.9% (w/v) sodium chloride with 1.7 U/ml heparin sodium and sterilized by filtration through a 0.22-μm nitrocellulose filter. Heparin sodium was purchased from APP Pharmaceuticals LLC (Schaumberg, IL). Pentobarbital was purchased from Ovation Pharmaceuticals, Inc. (Deerfield, IL), and penicillin G procaine was purchased from Phoenix Pharmaceuticals, Inc. (St. Joseph, MO). Isoflurane was purchased from Halocarbon Products (North Augusta, SC). β-Funaltrexamine and DAMGO were purchased from Sigma-Aldrich (St. Louis, MO), and solutions were prepared fresh as needed in sterile 0.9% (w/v) sodium chloride or assay buffer. [^{35}S]GTPγS (1250 Ci/mmol) was purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA). Other chemicals used were reagent grade from Sigma-Aldrich or Fisher Scientific (Pittsburgh, PA).

Drug Administration

Intravenous Heroin Administration. Heroin (30–100 μg/kg) was administered intravenously in a volume of 0.7 ml/kg body weight in sterile 0.9% NaCl (w/v) 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.

Intracranial β-FNA or Saline Administration. β-FNA or saline was administered into the lateral amygdala in a volume of 1 μl per side at a flow rate of 0.2 μl/min through a 32-gauge injection cannula (Plastics One) connected to a Hamilton microsyringe with polyethylene tubing. The injection cannulas were left in place for 10 min after β-FNA administration to allow for pressure equilibration. Infusions were administered using a microsyringe infusion pump (KDS Scientific, Boston, MA). All rats used for allodynia and self-administration studies received bilateral injections of β-FNA or saline, whereas rats used for determination of DAMGO-stimulated [^{35}S]GTPγS binding received unilateral injections.

Behavioral Procedures

Determination of Paw-Withdrawal Threshold (PWT). PWT was determined using the up-down method with von Frey filaments ranging in strength from 0.4 to 60 g.[21] Mechanical allodynia (PWT ≤4.0 g) was verified beginning 5–7 days after SNL surgery and at regular intervals throughout the self-administration studies (twice per week). PWT was also determined in sham-operated subjects at similar time points.

Heroin 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 houselight located outside the operant chamber, 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. Heroin self-administration sessions were conducted as reported previously.[5] Lever presses were engendered and maintained by infusion of 60 μg/kg heroin in 0.2 ml 0.9% saline with 1.7 U/ml heparin sodium over 6.2 s. Rats were placed in operant chambers, and illumination of the stimulus light above the lever indicated drug availability. Initially, each lever press resulted in delivery of an infusion of heroin (fixed-ratio 1). Once the number of infusions per session became stable (five successive sessions, in which the number of infusions earned did not vary by more than 10% from the mean), the fixed-ratio value was increased to 2. Each infusion was signaled by turning off the stimulus light above the lever, sounding the tone, and illumination of the houselight for 20 s. Lever presses had no programmed consequences during this time-out period. At the end of the time-out, the tone and houselight were turned off and the stimulus light above the lever was illuminated to indicate drug availability. Sessions were 4 h in duration and were conducted on weekdays only to prevent the development of physical dependence and tolerance.

Behavioral Effects of Heroin after Intraamygdala Administration of β-FNA or Saline

Antiallodynia. The presence of allodynia was verified for each animal 10–14 days after SNL and implantation of intravenous catheters as described above. Saline (n = 7) or β-FNA (2.5 nmol, n = 6) was injected into the amygdala (as described above), and the dose-effect curve for heroin in reversing the effects of SNL on PWT was determined 24 h later. Rats were acclimated to the testing chambers for at least 30 min, and baseline PWTs were determined. Each animal was then infused intravenously with 0.2 ml 0.9% (w/v) saline, and PWT was determined after 3 min. One hour later, baseline PWT was determined again, and each animal was infused with 30 μg/kg heroin and PWT was determined after 3 min. One hour later, baseline PWT was determined again to ensure that there was no residual drug effect, and the effect of 60 μg/kg heroin on PWT was determined in a similar manner. After 1 h, the effect of 100 μg/kg heroin was determined similarly. For animals treated with β-FNA, the dose-effect curve for the antiallodynic effects of heroin was determined before β-FNA administration and 1, 4, 7, 11, 14, and 17 days after β-FNA treatment. For saline-treated animals, the dose-effect curve for heroin was determined before and 24 h after saline treatment.

Self-administration. Rats were trained to self-administer infusions of 60 μg/kg heroin (as described in the Heroin self-administration section under Behavioral Procedures above) after 10–14 days after SNL or sham surgery and implantation of intracranial guide cannulas and intravenous catheters. Once the number of infusions was stable at fixed ratio 2 according to the same criteria given above, separate groups of rats (n = 6–7) were administered either saline or β-FNA (0.1, 0.3, 0.5, 1.0, or 2.5 nmol). Self-administration sessions were then conducted 24 h after intraamygdala treatment and daily thereafter for up to 17 days after intracranial treatment.

Histologic Verification of Cannula Placement

Animals were decapitated under isoflurane anesthesia, and the brains were rapidly removed and frozen in isopentane (−35°C) and stored at −80°C until sectioned. Coronal sections (20 μm) were thaw-mounted onto microscope slides and dessicated overnight at 4°C. Slides were then stored at −80°C until stained according to the methods of Kluver and Barrera (1953).[22] Cannula placement was verified by light microscopy in serial sections.

Data Analysis

The effect of β-FNA and saline injection into the amygdala on DAMGO-stimulated [^{35}S]GTPγS binding to sections was analyzed by one-way ANOVA, with net stimulation serving as the dependent measure, comparing the injected (ipsilateral) and noninjected (contralateral) side. The analysis was performed separately on the data obtained from the saline- and β-FNA-treated rats. The analysis was performed using JMP software version 5.0.1a (SAS Institute, Cary, NC).

The effects of intraamygdala β-FNA treatment on the antiallodynic effects of heroin over time were assessed using a 3 × 6 ANOVA design, with heroin dose (30, 60, or 100 μg/kg) and time after β-FNA treatment (repeated measures 1, 4, 7, 11, 14, and 17 days) serving as fixed factors and difference in paw withdrawal threshold before β-FNA treatment serving as the dependent measure. Difference scores from baseline values (before β-FNA treatment) were calculated with 95% confidence intervals using the 3 × 6 model, and lack of overlap with zero difference was considered statistically significant. Analysis was performed using SPSS software version 17.0 (SPSS, Inc., Chicago, IL). The effect of intraamygdala saline treatment on the antiallodynic effects of heroin was analyzed by two-way ANOVA, with heroin dose and time after saline administration (before *vs.* 24 h after) serving as the independent variables and PWT serving as the dependent measure. This analysis was performed using JMP software (SAS Institute).

The effect of β-FNA treatment on heroin self-administration was analyzed by two-way ANOVA, with SNL *versus* sham ligation being one independent variable and β-FNA dose being the other independent variable. The number of infusions of heroin self-administered after β-FNA treatment served as the dependent measure. β-FNA effects were examined for dose responsiveness in either sham-operated or SNL rats using one-way ANOVA, with dose of β-FNA serving as the independent variable and number of infusions of heroin per hour serving as the dependent measure. *Post hoc* analyses were performed using Bonferroni-Dunn *t* test for multiple comparisons, with the group administered saline serving as

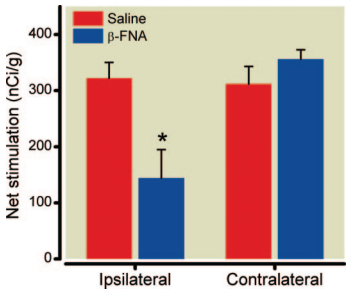

Fig. 1. Effect of β-FNA on DAMGO-stimulated [^{35}S]GTPγS binding in the lateral amygdala. The net stimulation of [^{35}S]GTPγS binding (nCi/mg) by [D-Ala^2,NMe-Phe^4, Gly-ol^5]enkephalin (DAMGO) is shown for the injected (ipsilateral) and uninjected (contralateral) lateral amygdala (mean ± SD). Rats were sacrificed 24 h after injection of either 2.5 nmol of β-funaltrexamine (β-FNA) or saline (1 μl). n = 8/group. *Significantly different from contralateral side, $P \leq 0.05$.

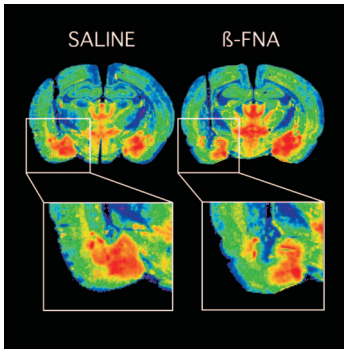

Fig. 2. Representative autoradiograms of DAMGO-stimulated [^{35}S]GTPγS binding. Representative autoradiograms of [D-Ala^2, NMe-Phe^4, Gly-ol^5]enkephalin (DAMGO)-stimulated [^{35}S]GTPγS binding are shown for sections taken from rats 24 h after administration of either saline (SALINE) or 2.5 nmol of β-funaltrexamine (β-FNA, β-FNA). The insert highlights the selectivity of the effect of β-FNA to the lateral amygdala.

the control. The effect of intraamygdala administration of 2.5 nmol of β-FNA on heroin self-administration over time was analyzed using a one-way ANOVA, with time after β-FNA administration serving as the independent variable and number of infusions of heroin administered serving as the dependent measure. A two-tailed P value of 0.05 or less was considered statistically significant. These analyses were performed using JMP software (SAS Institute).

Results

Effects of Intraamygdala β-FNA on DAMGO-stimulated [^{35}S]GTPγS Binding in Brain Sections

Injection of 2.5 nmol β-FNA into the lateral portion of the amygdala decreased net DAMGO stimulation of [^{35}S]GTPγS binding by 59.6 ± 14.3% (mean ± SD), compared with the contralateral side, whereas saline administration had no significant effect (fig. 1). Neither saline nor β-FNA administration into the lateral amygdala affected basal [^{35}S]GTPγS binding. In saline-treated rats, basal [^{35}S]GTPγS binding was 244 ± 23 nCi/mg on the saline-injected side, compared with 271 ± 32 nCi/mg on the contralateral side. In β-FNA-treated rats, basal [^{35}S]GTPγS binding was 278 ± 33 and 288 ± 24 nCi/mg on the β-FNA-injected and contralateral sides, respectively. The area of irreversible inhibition was confined to the lateral portion, with minimal effects seen in the central amygdala (fig. 2). The rostrocaudal spread of the effect of β-FNA was similar to the lateral spread from the injection site (data not shown).

Effects of Intraamygdala β-FNA on the Antiallodynic Effects of Intravenous Heroin

Heroin produced a robust antiallodynic effect in SNL rats that was dose dependent [$F(2,17) = 12.0$, $P = 0.0008$] (fig. 3). Administration of saline intravenously did not alter PWT in groups of rats administered either saline (2.9 ± 0.8 before, 2.3 ± 0.5 24 h after) or β-FNA (2.7 ± 0.3 before, 2.8 ± 0.4 24 h after) into the amygdala ($P > 0.05$). Administration of β-FNA into the lateral amygdala did not alter baseline PWT at any time point after administration, as well ($P > 0.05$; data not shown). β-FNA significantly attenuated the antiallodynic effects of all doses of heroin 24 h after administration into the lateral amygdala [$F(2,35) = 6.1$, $P = 0.006$] (fig. 3). Administration of saline into the lateral amygdala had no effect on the antiallodynic actions of any dose of heroin [$F(2,41) = 0.2$, $P = 0.9$] (fig. 3). There was a significant interaction between time after β-FNA administration and heroin dose on paw-withdrawal threshold ($P < 0.0001$). The effect of 30 μg/kg heroin returned to normal by day 4 after β-FNA treatment, whereas the effects of 60 and 100 μg/kg returned to pre-β-FNA treatment values by days 7 and 11,

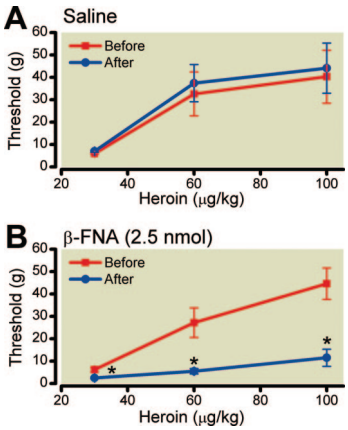

Fig. 3. Effect of intraamygdala β-FNA on the antiallodynic effects of heroin. Saline (*A*) or 2.5 nmol of β-funaltrexamien (β-FNA) (*B*) was injected bilaterally into the lateral amygdala, and heroin was administered intravenously 24 h after treatment. Paw-withdrawal threshold (PWT) was determined using von Frey filaments before and 3 min after injection of each dose of heroin (mean and 95% confidence intervals are shown). n = 7 (saline) and 6 (β-FNA). *Significantly different from PWT obtained before β-FNA or saline treatment, $P \leq 0.05$.

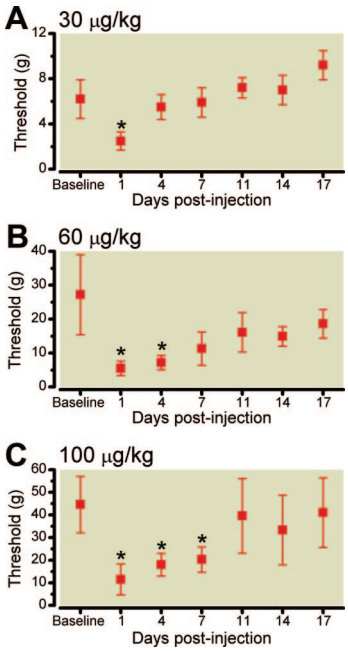

Fig. 4. Time course of β-FNA inhibition of the antiallodynic effects of heroin. The dose-effect curve for the antiallodynic actions of heroin were determined at the days shown after intraamygdala treatment with 2.5 nmol of β-funaltrexamine (β-FNA) using the same animals administered β-FNA that were used for the data obtained in figure 3. Mean and 95% confidence intervals are shown before (Baseline, BSL) and after β-FNA treatment for 30 (*A*), 60 (*B*), or 100 (*C*) μg/kg heroin. *Significantly different from BSL, $P \leq 0.05$.

respectively (fig. 4). Guide cannula placements are shown in fig. 5 for these rats.

Effects of Intraamygdala β-FNA on Heroin Self-administration

SNL significantly reduced the PWT threshold in the animals used for heroin self-administration relative to sham surgery

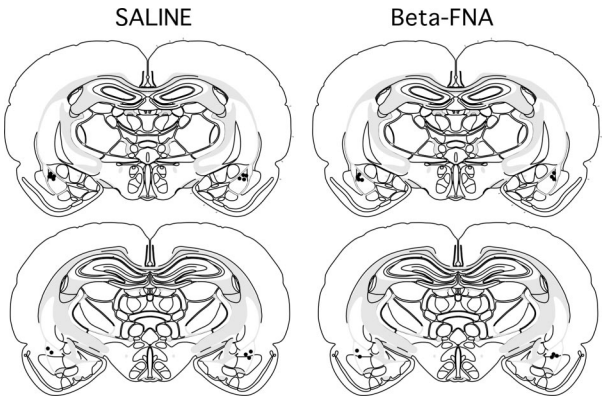

Fig. 5. Cannula placements for β-funaltrexamine (β-FNA) or saline treatment in animals used for determination of antiallodynic effects of heroin after intraamygdala injections. The cannula placements are shown for animals used to obtain the data presented in figures 3 and 4.

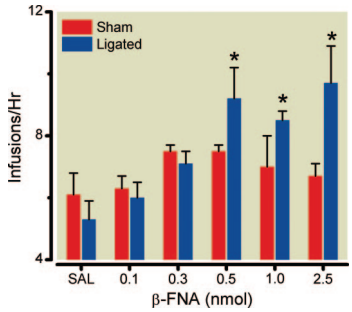

Fig. 6. Effects of intraamygdala β-FNA on heroin self-administration. B-funaltrexamine (β-FNA) or saline (SAL) was injected bilaterally into the lateral amygdale, and rats were allowed to self-administer infusions of 60 μg/kg heroin beginning 24 h later (mean ± SD). n = 5–7/group. *Significantly different from saline treatment, $P \leq 0.05$.

[F(1,61) = 124, $P < 0.0001$], and injection of either β-FNA or saline into the amygdala did not affect PWT in either the SNL [F(5,61) = 0.3, $P = 0.9$] or sham-operated group [F(5,59) = 1.0, $P = 0.4$]. The mean PWT (mean ± SD) after SNL for rats used for self-administration studies was 2.8 ± 0.6 g before β-FNA injection into the amygdala and 2.5 ± 0.6 g after injection. The mean ± SD PWT for the sham-operated group was 36.0 ± 6.7 and 33.6 ± 7.8 g before and after β-FNA treatment, respectively.

Heroin (60 μg/kg) maintained rates of self-administration that were comparable across all groups used for intraamygdala injection of saline or β-FNA, and there were no significant differences between any of these groups for behavior maintained by heroin before intracranial injection [F(11,61) = 1.0, $P = 0.5$]. We have previously demonstrated that rates of self-administration of this dose of heroin does not differ between SNL and sham-operated rats.[5] For the sham-operated group, injection of β-FNA (0.1–2.5 nmol) into the amygdala had no significant effect, compared with saline [F(5,29) = 2.1, $P = 0.1$] (fig. 6). Two animals in the sham-operated group were excluded due to incorrect cannula placement. Injection of β-FNA into the lateral amygdala produced a dose-dependent increase in heroin self-administration in rats after spinal nerve ligation [F(5,31) = 4.2, $P = 0.006$] (fig. 6). The effect of 0.5, 1.0, and 2.5 nmol was significantly different from that of saline in the spinal nerve-ligated groups ($P \leq 0.05$). Analysis of the self-administration data obtained 24 h after saline or β-FNA treatment by two-way ANOVA found a significant main effect [F(11,61) = 3.3. $P = 0.002$] and a significant interaction between β-FNA dose and ligation surgery (sham *vs.* SNL) [F(5,61) = 2.5, $P = 0.04$], with the effect of β-FNA being greater in the SNL *versus* sham-operated group ($P \leq 0.05$). The number of infusions of 60 μg/kg heroin self-administered in SNL rats remained increased for 9.2 ± 2.4 or 11.2 ± 2.3 days after administration of 0.5 or 1.0 nmol β-FNA into the lateral amygdala, respectively. Heroin self-administration was increased for 17.5 ± 4.8 days after injection of 2.5 nmol of

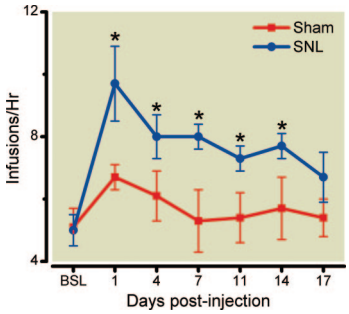

Fig. 7. Effects of intraamygdala injection of β-FNA (2.5 nmol) in SNL and sham rats with time. Number of infusions of heroin (60 μg/kg) self-administered hourly for groups of spinal nerve-ligated (SNL; n = 7) or sham (n = 7) rats injected with 2.5 nmol of β-funaltrexamine (β-FNA) are shown at selected days after β-FNA treatment (mean ± SD). *Significantly different from baseline (BSL) data, $P \leq 0.05$.

β-FNA into the amygdala of SNL rats, compared with pretreatment values. The number of hourly infusions of heroin self-administered before and during selected days after β-FNA (2.5 nmol) treatment is shown in figure 7. In sham-operated rats, there was no significant effect of 2.5 nmol of β-FNA with time [$F(13,97) = 0.8$, $P = 0.6$]; however, the effect of 2.5 nmol of β-FNA in SNL rats was time dependent [$F(13,97) = 3.5$, $P = 0.0002$]. The guide cannula placements are shown in figure 8 for all rats used in the self-administration studies.

Discussion

The current study suggests that MORs in the lateral portion of the amygdala are important for mediating heroin self-administration after SNL in rats, but not in sham-operated subjects. Stimulation of MOR in this region also appears to be at least partially responsible for the antiallodynic effects of opioids in SNL rats, as β-FNA injection produced a downward shift in the dose-effect curve for heroin, consistent with noncompetitive antagonism. These data implicate the lateral part of the amygdala as an important brain area, through which opioids reverse mechanical hypersensitivity and maintain self-administration selectively within the context of neuropathic pain.

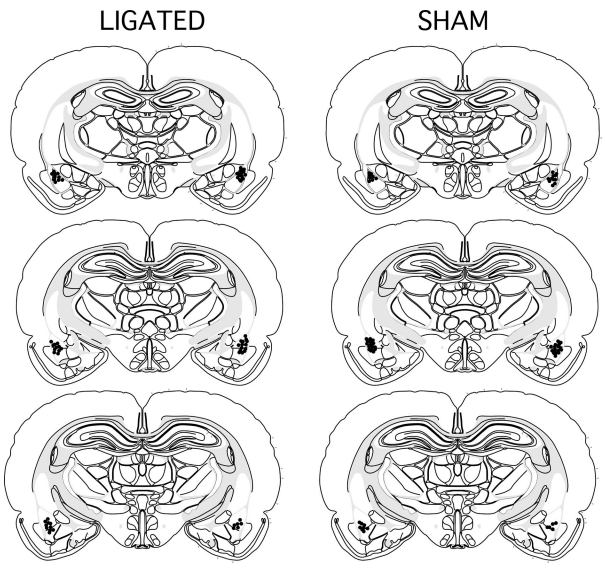

Fig. 8. Cannula placements for β-funaltrexamine (β-FNA) and saline treatment in animals used for heroin self-administration studies following intraamygdala injections. The cannula placements are shown for spinal nerve-ligated (LIGATED) or sham-operated (SHAM) animals used to obtain the data presented in figures 6 and 7.

The neuroanatomy of MOR within the amygdala suggests a number of potential neuronal populations that might be important for the maintenance of heroin self-administration in the presence of peripheral nerve injury. Opioids inhibit glutamate release in the lateral amygdala of rats after hind-paw injection of formalin and block-conditioned place aversion induced by this model of inflammatory pain.[23] MOR activation in the lateral amygdala also inhibits GABAergic projections to the CeA.[24] The CeA sends excitatory projection neurons to the ventrolateral PAG that are also inhibited by opioids.[9] The neurons projecting to the PAG from the central amygdala, in turn, activate neurons projecting to the RVM that stimulate descending inhibitory fibers that produce antinociception.[25] Therefore, excitatory output of the CeA to the PAG increases activation of descending inhibition through the RVM. Stimulation of the lateral amygdala by glutamatergic inputs increases the firing of GABAergic fibers that project to the central amygdala, thereby decreasing descending inhibition of spinal sites through the PAG-RVM pathway. Therefore, inhibiting excitation within the lateral amygdala should produce an increase in descending inhibition through the central amygdala-PAG-RVM pathway. This is the proposed mechanism by which opioids elicit analgesia through the lateral amygdala and provide a rationale for diminished antiallodynic effects of heroin produced by injection of β-FNA into this region. The self-administration data suggest that inhibition of this circuitry at the level of the lateral amygdala is a key mechanism by which opioids produce reinforcement in the presence of pain. However, this circuitry does not appear to be involved in opioid reinforcement in the absence of chronic pain, given that β-FNA injected into the lateral amygdala did not alter heroin self-administration in sham-ligated rats. Although differential involvement of pain-related circuitry has been suggested to underlie complex behavioral effects of opioids in the presence of chronic pain, the current study identifies a specific region that is differentially modulated in SNL and sham-operated rats related to the maintenance of self-administration, in addition to reflexive withdrawal from a mechanical stimulus. Identifying the neurotransmitters and/or neurotransmitter receptors and neuronal subtypes within the lateral amygdala that are regulated by opioids during self-administration specifically in SNL rats could lead to strategies that enhance opioid analgesia in the presence of

chronic pain without enhancing abuse liability in the absence of chronic pain, as MOR activation within the lateral amygdala appears to have distinct roles within these two contexts.

An important question to address with drug self-administration in the presence of pain is whether such studies model addiction, pain control, or some combination of both. Previous work suggests that SNL rats acquire heroin self-administration, at least in part, to alleviate mechanical hypersensitivity.[5] The current study further indicates that stimulation of MOR in the lateral amygdala is more important for maintaining heroin self-administration in the presence of chronic pain than in the absence of chronic hypersensitivity. However, given the myriad of connections between the lateral amygdala and limbic forebrain regions, it should not be concluded that heroin self-administration in SNL rats is solely mediated by reversal of mechanical hypersensitivity. Previous studies have shown that intrathecal administration of adenosine reverses mechanical hypersensitivity, but fails to alter opioid self-administration in SNL rats.[5] Further, the antiallodynic effects of heroin after intraamygdala administration of 2.5 nmol of β-FNA returned to pretreatment levels at previous time points than heroin intake by self-administration. Others have suggested that noxious stimulation depresses limbic activity, and that administration of opiates or other analgesics restores the activity of limbic forebrain systems to a normal state.[26,27] To this end, the lateral amygdala has important connections with limbic regions that comprise classic drug-reinforcement circuitry. The basolateral amygdala sends excitatory glutamatergic projections to the nucleus accumbens and medial prefrontal cortex (mPFC), which are thought to synapse directly on dopaminergic nerve endings.[10,11] Activation of these neurons would therefore be postulated to produce drug reinforcement. An amygdalonigral pathway has also been described using anterograde labeling of projections from the CeA to the substantia nigra as well as the ventral tegmental area.[12] The CeA sends inhibitory GABAergic fibers to the ventral tegmental area, which modulate the firing of dopaminergic neurons that project to the nucleus accumbens and ventral pallidum.[13,14] Stimulation of these fibers would therefore be postulated to decrease drug reinforcement. Noxious stimulation decreases the activity of dopaminergic neurons within the ventral tegmental area in humans, which likely results in diminished activity in forebrain projection neurons.[28] If the neuronal connections between these regions that are being modulated by self-administered opioids in the presence and absence of pain could be identified, it may be possible to understand the neurobiological basis of how pain alters drug-reinforcement mechanisms and develop rational strategies for increasing analgesic efficacy without increasing, or perhaps even decreasing, abuse liability. Further study of how opioids regulate amygdala function in the presence of pain may provide novel targets for chronic pain treatment.

The BLA sends projections to the prefrontal cortex, and activity within this circuit is associated with affective-motivational aspects of chronic pain as well as cognitive impairment.[28,29] Induction of arthritis by complete Freund adjuvant in rats results in a disruption of learning in a reinforcement paradigm, in which rats choose between large rewards that occur with low probability and small rewards that occur with high probability.[29] The Iowa Gambling Task is a similar paradigm in which human subjects learn to make responses that lead to rewards of lower value, but with higher probability of reinforcement, and learning within this paradigm requires an intact prefrontal cortex and amygdala in humans.[30,31] Interestingly, chronic pain patients display similar behavioral deficits with the Iowa Gambling Task as patients with lesions of the prefrontal cortex or amygdala.[32] Induction of chronic inflammatory arthritis in the knee of rats produces a specific alteration in the control of prefrontal cortical activity by the BLA, and this alteration is associated with cognitive disruption in a choice paradigm resembling the Iowa Gambling Task.[29] Specifically, the presence of chronic pain did not influence excitatory input to the mPFC from the amygdala, but did selectively increase inhibitory influence of the BLA inputs on mPFC neurons.[28] This effect was blocked by administration of corticotrophin-releasing factor type 1 antagonists into the BLA. Therefore, the presence of chronic pain selectively alters the influence of the BLA on mPFC activity, and these alterations are associated with cognitive and affective responses to pain. The current data suggest that altered BLA activity influences opioid-reinforcement mechanisms in the presence of pain, and projections to the mPFC from the BLA is one potential pathway that may be involved.

It is worth noting that the pharmacology of opioids in maintaining self-administration could differ, depending upon the operant paradigms employed. Numerous operant paradigms have been devised and studied over the past several decades, and a thorough presentation of the features of each are beyond the scope of this discussion. Although the fixed-ratio paradigm employed in the current study is rather easily conceptualized (a fixed number of lever presses are required for drug infusion with free access within the session), other paradigms employing variable ratios or fixed and variable time intervals have proven useful for examining certain aspects of drug reinforcement.[33,34] Progressive ratio schedules have been employed to determine how many responses a subject will emit for drug infusions; however, these schedules have typically proven of limited value for studying opioids.[35] Several operant paradigms have been devised to study impulsivity traits as well, although these have typically not been used for drug self-administration experiments.[36] Exploration of the effects of chronic pain on opioid self-administration using different operant paradigms could provide insights on specific aspects of reinforcement, such

as impulsivity or reinforcing efficacy, and complement the current study that has measured rate of heroin intake using a simple operant task.

In summary, MORs within the lateral portion of the amygdala are important for opioids in reversing mechanical hypersensitivity after peripheral nerve injury and in drug intake through self-administration, specifically in the context of neuropathic pain. Three distinct output systems from the lateral amygdala are potentially involved. One system is the ascending dopaminergic projections from the ventral tegmental area to limbic forebrain areas classically identified as important for drug reinforcement. Another system is the descending modulation of nociception that involves the PAG and RVM. Finally, the control of the prefrontal cortex by the lateral amygdala has been shown to be specifically altered within the context of pain and to produce alterations in complex cognitive behaviors. Future research will be aimed at determining the relative involvement of these systems in the maintenance of heroin self-administration in rats with neuropathic pain.

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ANESTHESIOLOGY REFLECTIONS

Preparing Local Anesthetics with Kersten's Apparatus

On December 21, 1920, Herbert R. Kersten of Boston was granted U.S. Patent No. 1,362,873 for his "Apparatus for Use in Preparing Anesthetics" (*above*). According to Kersten, prior to his invention, "pills" of dry local anesthetics were dissolved in solutions "brought to boil" in fragile glass or porcelain dishes which were flat enough to waste these expensive agents. He designed his apparatus with a metal cup with a "conical bottom . . . to concentrate the dregs . . . without requiring tilting or other manipulation of the dish" and to facilitate the syringing out of "expensive" local anesthetic as needed. Kersten soon ran a small business empire selling nothing but "dental cups" and his namesake apparatus out of a 6th-floor office of Boston's upscale Washington Building. Components of Kersten's Apparatus may have been scavenged from or supplied by his original office mate, a watch-case repairman! (Copyright © the American Society of Anesthesiologists, Inc. This image also appears in the *Anesthesiology Reflections* online collection available at www.anesthesiology.org.)

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