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Effects of Kappa Opioids in An Assay of Pain-Depressed Intracranial Self-Stimulation in Rats

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Abstract

Rationale—Selective, centrally acting kappa opioid agonists produce antinociception in a wide range of preclinical assays, but these compounds perform poorly as analgesics in humans. This discrepancy may be related to the behavioral depressant effects of kappa agonists. Kappa antagonists do not typically produce antinociception, but they produce antidepressant-like effects in some preclinical assays.

Objective—To test the hypothesis that the kappa agonist U69,593 and the kappa antagonist norbinaltorphimine would produce pronociceptive and antinociceptive effects, respectively, in an assay of pain-depressed behavior.

Methods—Effects of U69,593 (0.056–0.56 mg/kg), norbinaltorphimine (10–32 mg/kg) and morphine (3.2 mg/kg) were evaluated on the stimulation of a stretching response and the depression of intracranial self-stimulation (ICSS) of the medial forebrain bundle produced in rats by a common noxious stimulus (intraperitoneal administration of dilute lactic acid).

Results—U69,593 produced a dose-dependent blockade of acid-stimulated stretching but only exacerbated acid-induced depression of ICSS. Thus, U69,593 produced antinociception in the assay of pain-stimulated behavior but pronociceptive effects in the assay of pain-depressed behavior. Norbinaltorphimine did not alter acid-stimulated stretching or acid-induced depression of ICSS. The mu opioid agonist morphine blocked both acid-stimulated stretching and acid-induced depression of ICSS.

Conclusions—These results support the hypothesis that prodepressant effects of kappa agonists may limit their clinical utility as analgesics. These results do not support the use of kappa antagonists to treat depressant effects of pain. These findings illustrate the potential value of using complementary assays of pain-stimulated and pain-depressed behaviors for preclinical evaluation of candidate analgesics.

Keywords

U69,593; norbinaltorphimine; morphine; kappa opioid agonist; kappa opioid antagonist; intracranial self-stimulation; pain; nociception; analgesia; antinociception

INTRODUCTION

Selective kappa opioid receptor agonists that readily cross the blood-brain barrier and produce centrally acting effects after systemic administration have been evaluated as

potential alternatives to morphine-like mu opioid agonists for the treatment of pain. In preclinical studies, these selective and centrally acting kappa agonists have been shown to produce antinociceptive effects in many assays of acute, inflammatory and neuropathic pain at doses that do not produce mu agonist-like untoward effects (Catheline et al. 1996; Gallant and Meert 2008; Seguin et al. 1995; Vonvoigtlander et al. 1983). This profile of activity has been sufficiently promising to encourage clinical evaluations, but these compounds have performed poorly as analgesics in humans due to limits in analgesic efficacy and/or the emergence of untoward effects including aversive subjective effects (Pande et al. 1996a; Pande et al. 1996b; Wadenberg 2003). Consequently, studies with selective, centrally acting kappa agonists illustrate the potential for discrepancies between preclinical and clinical findings with candidate analgesic drugs.

The imperfect predictive validity of preclinical assays of antinociception has been a topic of considerable discussion (Blackburn-Munro 2004; Mogil 2009; Negus et al. 2006; Vierck et al. 2008; Whiteside et al. 2008). We have suggested that one source of suboptimal predictive validity may be rooted in the reliance of most preclinical assays on measures of “pain-stimulated” behaviors, which we define as behaviors that *INCREASE* in rate, frequency or intensity after delivery of a noxious stimulus (Negus et al. 2006; Stevenson et al. 2006). Common examples include withdrawal behaviors from escapable thermal or mechanical stimuli and stretching/flinching responses induced by inescapable chemical stimuli. An inherent vulnerability of such procedures is that expression of the target behavior can be reduced not only by manipulations that reduce sensory sensitivity to the noxious stimulus (i.e. true analgesia) but also by manipulations that impair motor function or other non-sensory processes necessary for expression of the target behavior (resulting in false-positive evidence for analgesia). This vulnerability is widely appreciated, and various strategies have been adopted to address it. We have advocated for an approach that incorporates assays of “pain-depressed” behavior, which we define as behaviors that *DECREASE* in rate, frequency or intensity after delivery of a noxious stimulus. Examples include pain-related depression of feeding, locomotion, or operant responding maintained by delivery of positive reinforcers (Flecknell 1994; Martin et al. 2004; Matson et al. 2007; Pereira Do Carmo et al. 2009b; Stevenson et al. 2006; Stevenson et al. 2009). In assays of pain-depressed behavior, analgesics increase rates of the target behavior, and as a result, drugs that impair motor function do not produce false-positive antinociceptive effects. Consequently, complementary use of assays that target both pain-stimulated and pain-depressed behaviors may facilitate dissociation of true analgesics from drugs that alter pain-related behaviors by producing non-specific effects. In addition, preclinical evaluation of drug effects on pain-depressed behaviors may facilitate prediction of drug effects on clinical assessments of pain-related functional impairment and depression of mood (Negus et al. 2006).

The purpose of the present study was to apply this principle to an assessment of selective, centrally acting kappa opioids. More specifically, this study compared effects of the kappa agonist U69,593 on pain-stimulated stretching and pain-depressed intracranial self-stimulation (ICSS) induced in rats by a commonly used noxious stimulus (intraperitoneal injection of a dilute acid solution). We hypothesized that U69,593 would produce the typical antinociceptive effect in the assay of acid-stimulated stretching. However, insofar as U69,593 also produces a variety of behavioral depressant effects, including a dose-dependent decrease in responding maintained by electrical brain stimulation in rats (Pereira Do Carmo et al. 2009a; Todtenkopf et al. 2004), we hypothesized that U69,593 would fail to produce antinociception in the assay of acid-depressed ICSS. The effects the kappa antagonist norbinaltorphimine (norBNI) were also evaluated. Although kappa antagonists do not produce antinociception in common assays of pain-stimulated behavior (Broadbear et al. 1994; Endoh et al. 1992; Portoghesi et al. 1987), they can produce antidepressant-like effects in preclinical procedures such as the forced-swim test, presumably by blocking

stress-induced enhancement of endogenous dynorphin release (Beardsley et al. 2005; Mague et al. 2003). Accordingly, we hypothesized that norBNI might block acid-induced depression of ICSS. The effects of U69,593 and norBNI were compared to the effects of the mu opioid agonist morphine, which blocked acid-induced stimulation of stretching and depression of ICSS in a previous study (Pereira Do Carmo et al. 2009b).

METHODS

Subjects

Twenty-four male Sprague-Dawley rats (Harlan, Frederick MD) weighing 297–334 g at the time of surgery were used for the studies of ICSS (n=11) and lactic-acid-induced stretching (n=13). Rats were individually housed and were maintained on a 12h light/dark cycle, with lights on from 6 a.m. to 6 p.m., Rats had free access to food and water except during testing. Animal maintenance and research were in compliance with NIH guidelines on care and use of animal subjects in research, and all animal use protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

Assay of Intracranial Self-Stimulation (ICSS)

Surgery—All rats were anesthetized with isoflurane gas (2.5–3% in oxygen; Webster Veterinary, Phoenix, AZ) for implantation of stainless steel electrodes (Plastics One, Roanoke, VA). One pole (the cathode) of each bipolar electrode was 0.25 mm in diameter and covered with polyamide insulation except at the flattened tip, whereas the other pole (the anode) was 0.125 mm in diameter and uninsulated. The cathode was implanted in the left medial forebrain bundle at the level of the lateral hypothalamus (2.8 mm posterior to bregma, 1.7 mm lateral from midsagittal suture, and 7.8 mm below dura). The anode was wrapped around one of three skull screws to serve as the ground, and the skull screws and electrode assembly were secured to the skull with orthodontic resin. The animals were allowed to recover for at least 7 days prior to commencing ICSS training.

Apparatus—Experiments were conducted in sound-attenuating boxes that contained modular acrylic test chambers (29.2 × 30.5 × 24.1 cm) equipped with a response lever (4.5 cm wide, extended 2.0 cm through the center of one wall, 3 cm off the floor), simulation lights (three lights colored red, yellow, and green, positioned 7.6cm directly above response lever), a 2-W white house light, and an ICSS stimulator (Med Associates, St. Albans, VT). Electrodes were connected to the stimulator via a swivel connector (Model SL2C, Plastics One, Roanoke, VA). The stimulator was controlled by a computer software program that also controlled all the programming parameters and data collection (Med Associates, St. Albans, VT).

Behavioral Procedure—After initial shaping of lever-press responding, rats were trained under a continuous reinforcement schedule of brain stimulation using procedures similar to those described previously (Carlezon and Chartoff 2007; Negus et al. in press; Pereira Do Carmo et al. 2009b). During sessions lasting 30 to 60 min, each lever press resulted in the delivery of a 0.5-s train of square wave cathodal pulses (0.1-ms pulse duration), and stimulation was accompanied by the illumination of the stimulus lights over the lever. Responses during the 0.5-s stimulation period did not earn additional stimulation. Initially, the frequency of stimulation was held constant at 126 Hz, and the stimulation intensity for each rat was adjusted gradually to the lowest value that would sustain a high rate of reinforcement (>20 stimulations/min). This intensity was then held constant for the remainder of the study, and frequency manipulations were introduced. Sessions involving frequency manipulations consisted of sequential 10-min components. During each component, a descending series of 10 current frequencies was presented, with a 60-s trial at

each frequency. The frequency range extended from 158–20 Hz in 0.10 log increments for initial studies in the group of rats tested with U69,593 and morphine. Because most responding under baseline conditions occurred at the higher frequencies, the subsequent study with norBNI in a second group of rats was conducted using a frequency range of 158–56 Hz in 0.05 log increments. Each frequency trial began with a 10-s time out during which responding had no scheduled consequences. During the last 5 s of this time out, 5 non-contingent “priming” stimulations were delivered at the frequency available during that trial, and the lever lights were illuminated during each stimulation. Non-contingent stimulations were separated by intervals of 0.5 s. This non-contingent stimulation was then followed by a 50-s “response” phase, during which responding produced electrical stimulation under the continuous reinforcement schedule. Training continued with presentation of up to six sequential components per day until rats reliably responded for the first three frequency trials of all components for at least two consecutive days. Eleven rats met this criterion and were used for ICSS studies described below. The remaining thirteen rats were assigned to assays of lactic acid-induced stretching.

Once training was completed, testing was initiated using one protocol in one group of 6 rats for studies with the kappa agonist U69,593 and the mu agonist morphine, and a modified protocol in a second group of 5 rats for studies with long-acting antagonist norBNI. U69,593 and morphine were studied using a protocol in which test sessions consisted of 6 sequential components. The first component of each test session was considered to be an acclimation component, and data from this component were discarded. Data from the second and third “baseline” components were used to calculate baseline parameters of frequency-rate curves for that session (see Data Analysis). Treatment manipulations were introduced during a 30-min time-out period after the third component, and this time out was followed by three additional “test” components lasting a total of 30 min. This testing period was chosen to match the session length for stretching studies (see below), and it should also be noted that our previous studies demonstrated that lactic acid produced sustained decreases in ICSS for up to 90 min [Pereira Do Carmo, 2009 #1852]. U69,593 (0–0.56 mg/kg IP) was tested as a pretreatment to lactic acid (0 or 1.5% IP in a volume of 1.0 ml/kg). U69,593 or its vehicle was administered 10 min before the test components, and lactic acid or its vehicle was administered 10 min later, immediately before the test components. U69,593 doses were delivered in a modified Latin-square order across rats, and each week, a rat was tested with a given U69,593 dose in combination with lactic acid vehicle on one test day and with 1.5% lactic acid on another test day. At the conclusion of studies with U69,593, effects of morphine were also evaluated for comparison in 5 of the same 6 rats (one rat lost its electrode and could not be tested further). Vehicle or 3.2 mg/kg IP morphine was administered 30 min before vehicle or 1.5% lactic acid. All other testing conditions were identical to those described above for U69,593. Test sessions were typically conducted on Tuesdays and Fridays. Training sessions, consisting of only the initial three components, were conducted on Mondays, Wednesdays and Thursdays.

NorBNI was studied in a different group of 5 rats using a protocol that spanned two days to accommodate the slow onset and long duration of selective kappa antagonist effects. More specifically, doses and pretreatment times were based on studies using the stretching procedure (see below) and on previous studies demonstrating that systemic administration of 5–40 mg/kg norBNI produces kappa antagonist effects that peak in selectivity and magnitude after approximately 24 hr and last for at least one week in rodents (Broadbear et al. 1994; Endoh et al. 1992; Friese et al 1997; Portoghesi et al. 1987; Takemori et al. 1988). Thus, on the first day ICSS studies with norBNI, rats were exposed to a baseline session consisting of only three components. As above, data from the first component were discarded, and data from the second and third components were used to calculate baseline parameters of frequency-rate curves (see Data Analysis). Immediately after this baseline

session, rats were treated with vehicle, 10 or 32 mg/kg norBNI IP. On the second day, 24 hr after vehicle or norBNI, rats were exposed to a test session consisting of six components, and rats were treated with 1.5% lactic acid (1.0 ml/kg IP) after the third component. Thus, the first three components were used to assess the effects of norBNI in the absence of a noxious stimulus, and the final three components were used to assess the effects of norBNI on acid-induced depression of ICSS. Vehicle, 10 and 32 mg/kg norBNI were tested in that order, with 18 days between the first and second dose of norBNI to permit dissipation of effects produced by the first dose.

Data Analysis—The primary dependent variable in this ICSS procedure was the reinforcement rate in stimulations/min during each frequency trial. To normalize these data, raw reinforcement rates from each trial were converted to Percent Maximum Control Rate (%MCR), with the maximum control rate defined as the mean of the maximal rates observed during the second and third “baseline” components for that session. Thus, %MCR values for each trial were calculated as (Response Rate During a Frequency Trial ÷ Maximum Control Rate) × 100. For each test session, data from the second and third components were averaged to yield a baseline frequency-rate curve, and data from the three test components were averaged to yield a test frequency-rate curve. Baseline and test curves were then averaged across rats to yield mean baseline and test curves for each manipulation. For statistical analysis, results were compared by two-way ANOVA, with treatment and ICSS frequency as the two factors. A significant ANOVA was followed by a Holman-Sidak post hoc test, and the criterion for significance was set at $p < 0.05$.

To provide an additional summary of ICSS performance, the total number of stimulations obtained at all frequencies was summed for each test component and averaged across the three test components of each experimental session in each rat. Data for Total Stimulations were then averaged across rats in each experimental condition and compared by one-way ANOVA or T-test as appropriate. A significant ANOVA was followed by the Dunnett post hoc test, and the criterion for significance was set a priori at $p < 0.05$.

Assay of Lactic Acid-Induced Stretching

Behavioral Procedure—Thirteen rats that failed to meet criteria for ICSS within 4 weeks were used for studies of lactic acid-induced stretching as described previously (Pereira Do Carmo et al. 2009b). Initial studies with U69,593 and morphine were conducted in a single group of six rats. During test sessions, rats were placed into an acrylic test chamber (31.0cm × 20.1cm × 20.0cm) for a 30 min observation period. U69,593 (0–0.56 mg/kg IP, 10 min pretreatment) or morphine (0 or 3.2 mg/kg IP, 30 min pretreatment) was administered prior to lactic acid (0 or 1.5% IP in a volume of 1.0 ml/kg). The observation period began immediately after acid injection. A stretch was operationally defined as a contraction of the abdomen followed by a stretching of the hind limbs, and the number of stretches during the observation period was counted. U69,593 doses were delivered in a modified Latin-square order across rats. Each week, a rat was tested with a given U69,593 dose in combination with lactic acid vehicle on one test day and with 1.5% lactic acid on another test day. Test sessions for any one rat were separated by at least 48 hr. Morphine was tested after all studies with U69,593 were completed.

Studies with norBNI were conducted in a separate group of seven rats using a protocol that spanned four days to accommodate the slow onset and long duration of selective kappa antagonist effects as described above. On the first day, rats were treated with vehicle or 10 mg/kg norBNI IP. On the second and fourth day, 24 hr or 72 hr after the initial treatment, rats were treated with lactic acid (1.5% IP in a volume of 1.0 ml/kg) 10 min after treatment with either vehicle or a maximally effective dose of U69,593 (0.56 mg/kg IP). The four-day

study with norBNI vehicle was conducted before the four-day study with 10 mg/kg norBNI. The order of treatment with U69,593 or its vehicle on the second and fourth days of each test period was counterbalanced across rats. This design permitted assessment of (1) any direct antinociceptive effects of norBNI alone (when rats were treated with U69,593 vehicle before lactic acid) and (2) the kappa antagonist effects of norBNI (when rats were treated with 0.56 mg/kg U69,593 before lactic acid).

Data Analysis—Drug effects on lactic acid-induced stretching were evaluated by one-way ANOVA or T test as appropriate. A significant ANOVA was followed by the Dunnett's or Newman-Keuls post hoc test, and the criterion for significance was set at $p < 0.05$.

Drugs

Lactic acid and U69,593 were purchased from Sigma Chemical Co. (St. Louis, MO). Morphine sulfate was provided by the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD). Norbinaltorphimine 2HCl was synthesized by Drs. Cheng and Rice. All solutions were prepared in sterile water for IP injection.

RESULTS

U69,593 and Morphine Effects in the Assay of Lactic Acid-Stimulated Stretching

The stretching response was not elicited by treatment with lactic acid vehicle administered alone or after pretreatment with any dose of U69,593 or morphine (data not shown). Figure 1 shows the effects of U69,593 and morphine on stretching elicited by IP lactic acid (1.5%). Lactic acid alone stimulated stretching. U69,593 produced a dose-dependent blockade of acid-stimulated stretching, and stretching stimulated by 1.5% lactic acid was significantly lower after 0.56 mg/kg U69,593 than after pretreatment with U69,593 vehicle. Pretreatment with 3.2 mg/kg morphine eliminated acid-stimulated stretching in all rats.

Effects of U69,593 and Morphine in the Assay of Lactic Acid-Depressed ICSS

Figure 2 shows the effects of the same noxious stimulus (IP injection of 1.5% lactic acid) on ICSS. During each test session, a "baseline" frequency-rate curve was determined before testing to permit determination of the maximum control rate (MCR) for that session. Over the course of the entire study in this group of rats, the average MCR was 33.4 ± 6.4 stimulations/trial. Reinforcement rates during each frequency trial of a session were then expressed as a percentage of that session's maximum control rate (%MCR), and the average baseline frequency-rate curve for all studies with U69,593 is shown in Figure 2 as a gray line. Rats generally did not respond at frequencies of 20–50 Hz, and reinforcement rates increased across a frequency range of 63–158 Hz. Maximum reinforcement rates were usually observed at the highest stimulation frequencies. Treatment with the lactic acid vehicle (sterile water) had little effect on the frequency-rate curve. However, treatment with 1.5% lactic acid depressed ICSS, producing a rightward and downward shift in the frequency-rate curve.

Figure 3 shows the effects of U69,593 on control and acid-depressed ICSS. When administered as a pretreatment to lactic acid vehicle, U69,593 produced a dose-dependent rightward and downward shift in the ICSS frequency-rate curve (Figure 3 left panel). A low dose of 0.056 mg/kg U69,593 had no effect on ICSS. However, 0.18 and 0.56 mg/kg U69,593 dose-dependently and significantly decreased rates of reinforcement at frequencies from 80–158 Hz. When U69,593 was administered as a pretreatment to 1.5% lactic acid, it produced a further, dose-dependent reduction in acid-depressed ICSS (Figure 3, center panel). The low dose of 0.056 mg/kg U69,593 decreased reinforcement rates relative to acid alone at 100 Hz. Higher doses of 0.18 and 0.56 mg/kg U69,593 decreased reinforcement

rates relative to acid alone at frequencies of 100–158 Hz. Overall, U69,593 produced a dose-dependent decrease in Total Stimulations whether it was administered before lactic acid vehicle or 1.5% lactic acid (Figure 3, right panel).

Figure 4 shows the effects of morphine on control and acid-depressed ICSS. As described above, pretreatment with 1.5% lactic acid alone depressed ICSS. In contrast to U69,593, a dose of 3.2 mg/kg morphine had no effect on ICSS in the absence of acid treatment, and it completely blocked acid-induced depression of ICSS.

Effects of norBNI

Figure 5 shows that pretreatment with 10 mg/kg norBNI alone did not significantly alter acid-stimulated stretching; however, this dose of norBNI was sufficient to significantly antagonize the antinociceptive effect of 0.56 mg/kg U69,593. Figure 6 shows that pretreatment with 10 and 32 mg/kg norBNI did not alter either control or acid-depressed ICSS. The mean $\pm$ SEM MCR in this group of rats for the entire study was 55.9 ± 3.3 stimulations/trial.

DISCUSSION

The main finding of this study was that the kappa agonist U69,593 produced antinociception in a conventional assay of pain-stimulated behavior but only pronociceptive effects in an assay of pain-depressed behavior in rats. The kappa antagonist norBNI, tested at doses that produce kappa antagonist and antidepressant-like effects, failed to alter either pain-stimulated or pain-depressed behaviors. Effects of both kappa opioids were distinct from those of morphine, which produced antinociception in assays of both pain-stimulated and pain-depressed behavior. Only one kappa agonist and one kappa antagonist were evaluated in this study, and the degree to which these findings might generalize to other kappa agonists and antagonists remains to be determined. However, these findings with highly selective kappa opioids provide new insights into effects that may limit the utility of selective, centrally acting kappa agonists for the treatment of pain and also suggest that kappa antagonists administered alone may not be useful for treating the prodepressant effects of pain. In addition, these results illustrate the value of using complementary assays of pain-stimulated and pain-depressed behavior to evaluate candidate analgesic drugs.

Effects of U69,593, morphine, and norBNI in the present assay of lactic acid-stimulated stretching are consistent with the effects of these compounds reported previously in other assays of pain-stimulated behaviors in mice, rats and nonhuman primates (e.g. Bartok and Craft 1997; Broadbear et al. 1994; Cook et al. 2000; Dykstra and Massie 1988; Gallantine and Meert 2008; Ko et al. 1999; Negus and Mello 2002; Seguin et al. 1995). For example, both U69,593 and morphine produced dose-dependent decreases in acetic acid-stimulated stretching in mice, whereas norBNI had no effect alone but produced selective kappa antagonist effects with a slow onset and a long duration of action (Broadbear et al. 1994).

The antinociceptive effects of centrally acting kappa agonists in the present assay of acid-stimulated stretching and in many previous studies of pain-stimulated behavior are qualitatively similar to effects produced by mu agonists. However, while morphine and other mu agonists are effective analgesics, centrally acting kappa agonists have failed to produce viable analgesia due either to insufficient efficacy or the emergence of dose-limiting untoward effects (Machelska et al. 1999; Mangel et al. 2008; Pande et al. 1996a; Pande et al. 1996b; Wadenberg 2003). For example, the kappa agonist enadoline produced significant analgesic effects in a study of post-surgical pain in humans, but adverse neuropsychiatric effects caused early termination of treatment, and enadoline did not produce significant analgesia in a separate study of dental pain (Pande et al. 1996a; Pande et

al. 1996b). Thus, research with centrally acting kappa agonists provides one example of the imperfect predictive validity of preclinical procedures used to assess candidate analgesic drugs (Blackburn-Munro 2004; Mogil 2009; Negus et al. 2006; Vierck et al. 2008; Whiteside et al. 2008).

One strategy to address this discordance between preclinical and clinical findings has been to develop and incorporate new measures of pain-related behavior that might increase predictive validity, and we have advocated for the inclusion of assays that assess pain-depressed behaviors (Negus et al. in press; Negus et al. 2006; Stevenson et al. 2006). Assays that evaluate drug effects on pain-depressed behaviors may confer at least two advantages to the practice of analgesic drug evaluation. First, antinociception is assays of pain-depressed behavior is expressed as an INCREASE in the target behavior, and as a result, drugs that produce non-selective motor impairment do not produce false-positive effects. Of course, assays of pain-depressed behavior may be vulnerable to drugs that produce non-selective stimulation of behavior; however, complementary use of assays of pain-stimulated and pain-depressed behavior may provide a framework for distinguishing between true analgesics (effective in both types of assays) from drugs that produce non-selective behavioral suppression (effective only in assays of pain-stimulated behavior) or non-specific stimulant effects (effective only in assays of pain-depressed behavior). Second, measures of pain-depressed behaviors are clinically relevant insofar as they serve as principle indicators of pain in veterinary medicine (Council 1996; 2003; Morton and Griffiths 1985) and as increasingly important complements to verbal reports of pain in human medicine (Cleeland and Ryan 1994; Dworkin et al. 2008). Moreover, pain-related depression of behavior in humans is often accompanied by a co-morbid depression of mood (Bair et al. 2003; Gureje et al. 2008; Jann and Slade 2007; Lepine and Briley 2004). Overall, the assessment of drug effects on pain-depressed behavior in preclinical studies may improve prediction of drug effects on pain-related depression of behavior and mood in clinical contexts.

In the present study, pain-depressed behavior was evaluated using an assay of lactic acid-induced depression of ICSS. As reported previously, morphine blocked acid-induced depression of ICSS at a dose and pretreatment time that also blocked acid-stimulated stretching but did not alter control ICSS (Pereira Do Carmo et al. 2009b). The ability of morphine to produce antinociception in assays of both pain-stimulated and pain-depressed behavior suggests that morphine blocked sensory detection of the noxious stimulus, and this interpretation is consistent with the analgesic efficacy of morphine. In contrast, U69,593 doses that blocked pain-stimulated stretching also reduced control ICSS and only exacerbated acid-induced depression of ICSS. These findings suggest that U69,593 did not block sensory detection of the noxious stimulus, but rather produced a non-selective depression of behavior and/or an enhancement of the prodepressant effects of pain. It is well-established that kappa agonists can produce sedation, loss of muscle tone and impaired motor function at doses similar to those producing antinociception in assays of pain-stimulated behavior (Dykstra et al. 1987; Gallantine and Meert 2008; Negus et al. 2008; Seguin et al. 1995), and these motor effects may have limited the ability of U69,593 to block acid-induced depression of ICSS in the present study. However, kappa agonists may also have interacted more directly with the prodepressant effects of pain on neural substrates that mediate ICSS. For example, ICSS of the median forebrain bundle is mediated at least in part by activation of mesolimbic dopamine neurons (Stellar and Stellar 1985), and both noxious stimulation and treatment with kappa agonists can inhibit activity within this mesolimbic dopaminergic system (Carlezon et al. 2006; Di Chiara and Imperato 1988; Margolis et al. 2003; Ungless et al. 2004). Overall, the results of the present study are consistent with the hypothesis that kappa agonists produce pronociceptive effects in an assay of pain-depressed behavior, and these pronociceptive effects may contribute to the limited value of kappa agonists as analgesics.

In the present study, the kappa antagonist norBNI did not block acid-induced depression of ICSS. This lack of effect cannot be attributed to inadequate dosing, because norBNI was tested at doses sufficient to block the effects of U69,593 in the assay of acid-stimulated stretching in this study, and sufficient to produce antidepressant-like effects in a forced-swim test procedure in a previous study in rats (Beardsley et al. 2005). Thus, although kappa receptor stimulation induced by exogenous kappa agonists is clearly sufficient to depress ICSS (present study, Todtenkopf et al. 2004), the present results indicate that kappa receptor stimulation was not necessary for acid-induced depression of ICSS. This in turn suggests that acid-induced depression of ICSS was not mediated by endogenous dynorphin release and subsequent activation of kappa opioid receptors, a mechanism that has been implicated in other types of stress-induced and depression-related behaviors in rodents (Mague et al. 2003; McLaughlin et al. 2003). More generally, these findings suggest that kappa antagonists administered alone may not be useful in treating the prodepressant effects of pain. However, the present study examined effects of a relatively short-acting noxious stimulus, and more sustained noxious stimulation such as that associated with chronic inflammatory or neuropathic pain may be more likely to engage dynorphin/kappa receptor pathways.

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Abbreviations

NorBNI	norbinaltorphimine
ICSS	intracranial self-stimulation

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Figure 1. U69,593 and morphine block acid-stimulated stretching

Abscissa: dose U69,593 in mg/kg, IP. Ordinate: Number of stretches observed during a 30-min observation period. Each bar shows mean $\pm$ SEM for 6 rats. For U69,593, one-way ANOVA indicated a significant main effect of U69,593 dose [$F(3,15)=$, $p=0.0156$].

*Asterisk indicates a significant difference from “Veh” as determined by the Dunnett’s post hoc test, $p<0.05$. For morphine, a paired t-test indicated a significant effect of morphine ($p<0.05$).

Figure 2. Lactic acid treatment depresses ICSS

Abscissa: Frequency of electrical brain stimulation in Hz (log scale). Ordinate: Percent maximum control response rate. The average baseline ICSS frequency-rate curve for the entire study in this group of rats is shown by the gray line for comparison, but these data were not included in statistical analysis. Effects of treatment with drug vehicle + lactic acid vehicle and drug vehicle + 1.5% lactic acid are shown by diamonds and circles, respectively. All points show mean $\pm$ SEM for 6 rats. In a comparison of ICSS frequency-rate curves after treatment with lactic acid vehicle and 1.5% lactic acid, there was a significant main effect of frequency [$F(9,100)=38.5$, $p<0.001$], a significant main effect of lactic acid treatment [$F(1,100)=16.1$, $p<0.001$], but not a significant frequency $\times$ treatment interaction [$F(9,100)=1.8$, $p=0.088$]. Filled circles indicate frequencies at which reinforcement rates after lactic acid were significantly lower than rates after vehicle treatment as determined by the Holman-Sidak post hoc test, $p<0.05$.

Figure 3. U69,593 fails to block acid-induced depression of ICSS

(a) The left and center panels show U69,593 effects on full frequency-rate curves. Abscissae: Frequency of electrical brain stimulation in Hz (log scale). Ordinates: Percent maximum control response rate. The left panel shows data for U69,593 administered as a pretreatment to lactic acid vehicle. There was a significant main effect of frequency [$F(9,200)=68.9$; $p<0.001$], a significant main effect of U69,593 dose [$F(3,200)=28.6$, $p<0.001$], and a significant frequency $\times$ dose interaction [$F(27,200)=4.1$; $p<0.001$]. The center panel shows data for U69,593 administered as a pretreatment to 1.5% lactic acid. There was a significant main effect of frequency [$F(9,200)=30.4$; $p<0.001$], a significant main effect of U69,593 dose [$F(3,200)=5.7$, $p<0.001$], but not a significant frequency $\times$ dose interaction [$F(27,200)=1.1$; $p=0.264$]. Filled symbols indicate frequencies at which reinforcement rates after U69,593 treatment were significantly lower than rates after vehicle alone (left panel) or acid alone (center panel) as determined by the Holman-Sidak post hoc test, $p<0.05$. (b) The right panel shows U69,593 effects on the total number of stimulations per component. Abscissa: Dose U69,593 in mg/kg. Ordinate: Total Stimulations per component. The asterisk indicates a significant lactic acid-induced depression of ICSS as determined by paired t-test ($p<0.05$). Dollar signs indicate a significant depression of ICSS by U69,593 as determined by significant one-way ANOVA in the respective lactic acid vehicle or 1.5% lactic acid treatment conditions, followed by the Dunnett post hoc test ($p<0.05$). All data show results from 6 rats.

Figure 4. Morphine blocks acid-induced depression of ICSS

(a) The left and center panels show morphine effects on full frequency-rate curves. Abscissae: Frequency of electrical brain stimulation in Hz (log scale). Ordinates: Percent maximum control response rate. The left panel shows data for morphine administered as a pretreatment to lactic acid vehicle. There was a significant main effect of frequency [$F(9,80)=144.0$; $p<0.001$], but not a significant main effect of morphine dose [$F(1,80)=0.01$, $p=0.912$] or a significant frequency $\times$ dose interaction [$F(9,80)=0.3$; $p=0.980$]. The center panel shows data for morphine administered as a pretreatment to 1.5% lactic acid. There was a significant main effect of frequency [$F(9,80)=144.0$; $p<0.001$], a significant main effect of morphine dose [$F(1,80)=14.7$, $p<0.001$] and a significant frequency $\times$ dose interaction [$F(9,80)=6.3$; $p<0.001$]. Filled symbols indicate frequencies at which reinforcement rates after morphine treatment were significantly higher than rates after acid alone (center panel) as determined by the Holman-Sidak post hoc test, $p<0.05$. (b) The right panel shows morphine effects on the total number of stimulations per component. Abscissa: Dose morphine in mg/kg. Ordinate: Total Stimulations per component. The asterisk indicates a significant lactic acid-induced depression of ICSS as determined by t-test ($p<0.05$). The dollar sign indicates a significant blockade of lactic acid-induced depression of ICSS as determined by t-test in the 1.5% lactic acid-treated condition ($p<0.05$). All data show results from 5 rats.

Figure 5. NorBNI does not block acid-stimulated stretching

Abscissa: Treatment condition. Ordinate: Number of stretches observed during a 30-min observation period. Each bar shows mean $\pm$ SEM for 6 rats. There was a significant effect of treatment [$F(3,18)=12.1$, $p<0.01$]. Asterisk indicates that stretching was significantly lower in the U69,593 + Lactic Acid condition than in all other conditions as determined by the Newman-Keuls post hoc test. In addition, the Lactic Acid alone group was not different from the NorBNI + Lactic Acid group or the NorBNI+U69,593+Lactic Acid group ($p>0.05$).

Figure 6. NorBNI fails to alter basal ICSS or acid-induced depression of ICSS

(a) The left and center panels show norBNI effects on full frequency-rate curves. Abscissae: Frequency of electrical brain stimulation in Hz (log scale). Ordinates: Percent maximum control response rate. The left panel shows data for norBNI administered as a pretreatment to lactic acid vehicle. There was a significant main effect of frequency [$F(9,120)=123.7$; $p<0.001$] and a significant main effect of norBNI dose [$F(2,120)=5.3$, $p=0.006$], but not a significant frequency $\times$ dose interaction [$F(18,120)=0.4$; $p=0.982$]. The center panel shows data for norBNI administered as a pretreatment to 1.5% lactic acid. There was a significant main effect of frequency [$F(9,120)=39.0$; $p<0.001$], but not a significant main effect of norBNI dose [$F(2,120)=2.1$, $p=0.12$] or a significant frequency $\times$ dose interaction [$F(18,120)=0.4$; $p=0.989$]. The effects of norBNI were not significantly different from the effects of vehicle alone (left panel) or acid alone (center panel) as determined by the Holman-Sidak post hoc test. (b) The right panel shows norBNI effects on the total number of stimulations per component. Abscissa: Dose norBNI in mg/kg. Ordinate: Total Stimulations per component. The asterisk indicates a significant lactic acid-induced depression of ICSS as determined by paired t-test ($p<0.05$). However, there was not a significant effect of norBNI on total stimulations during either the lactic acid vehicle or 1.5% lactic acid treatment conditions as determined by one-way ANOVAs. All data show results from 5 rats.