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Differential tolerance to morphine antinociception in assays of pain-stimulated vs. pain-depressed behavior in rats ☆

Ahmad A. Altarifi [a,b,*], S. Stevens Negus [b]

[a] Department of Pharmacology, School of Medicine, Faculty of Medicine, Jordan University of Science and Technology, P.O.Box 3030, Irbid 22110, Jordan
[b] Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, USA

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ABSTRACT

In preclinical research on pain and analgesia, noxious stimuli can stimulate expression of some behaviors (e.g. withdrawal reflexes) and depress others (e.g. feeding, locomotion, and positively reinforced operant responding). Tolerance to morphine antinociception is a robust and reliable phenomenon in preclinical assays of pain-stimulated behavior, but development of morphine tolerance in assays of pain-depressed behavior has not been studied. This study compared morphine antinociceptive tolerance in parallel assays of pain-stimulated and pain-depressed behavior in male Sprague–Dawley rats. Intraperitoneal injection of dilute lactic acid served as a noxious stimulus to stimulate a stretching response in one group of rats and to depress operant responding for electrical brain stimulation (intracranial self-stimulation; ICSS) in another group of rats. Antinociception produced by morphine (1.0 mg/kg) was determined after a regimen of chronic treatment with either saline or morphine in separate subgroups of rats in each procedure. In rats receiving chronic saline, acid alone stimulated a stretching response and depressed ICSS, and both acid effects were blocked by 1.0 mg/kg morphine. Rats receiving chronic morphine displayed hyperalgesic responses to the acid noxious stimulus in both procedures. Complete tolerance developed to morphine antinociception in the assay of acid-stimulated stretching, but morphine retained full antinociceptive effectiveness in the assay of acid-depressed ICSS. These results suggest that morphine antinociception in an assay of pain-depressed behavior is relatively resistant to tolerance. More broadly, these results suggest that antinociceptive tolerance can develop at different rates or to different degrees for different measures of antinociception.

1. Introduction

Preclinical assays of nociception play a key role in research on both the neurobiology of pain and the development of novel analgesics. Sensitivity of these procedures to antinociceptive effects of mu opioid analgesics like morphine is important for claims of translational relevance, because opioids are among the most effective analgesics for pain treatment in humans (Max, 2003). In addition, these procedures are often used to investigate variables that influence expression of opioid antinociception and that might also modulate opioid analgesia. For example, a common finding in many preclinical procedures is the development of tolerance to opioid antinociception after regimens of repeated opioid treatment (Fernandes et al., 1977a, 1977b; Williams et al., 2013). This antinociceptive tolerance is typically viewed as an undesirable effect, and a large literature has been devoted to strategies for reducing opioid antinociceptive tolerance with the underlying rationale that reduction of tolerance would improve clinical utility (Garzon et al., 2008; Ueda and Ueda, 2009). However, there is weaker evidence from clinical studies to suggest that tolerance is a significant obstacle to the use of mu agonists to treat pain (Foley, 1995; Rosenblum et al., 2008). Although analgesic tolerance can occur, pain can be effectively managed in many patients with little or no change in opioid dose over time, and dose escalation is often attributed to factors other than pharmacodynamic tolerance, such as disease progression. Moreover, tolerance to opioid side effects such as sedation, nausea/emesis, and respiratory depression can improve the safety and tolerability of mu agonists for the treatment of pain (Benyamin et al., 2008; Labianca et al., 2012).

These observations suggest a potential discordance between the preclinical phenomenon of opioid antinociceptive tolerance and the clinical phenomenon of opioid analgesic tolerance. One potential basis for this discordance could be related to the dependent measures of pain and analgesia in preclinical vs. clinical studies. In human clinical contexts, the principal measure of pain is a verbal report, such as a visual analog scale (Hawker et al., 2011; Rauh et al., 2013; Schmitter

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* Corresponding author at: Department of Pharmacology, School of Medicine, Faculty of Medicine, Jordan University of Science and Technology, P.O.Box 3030, Irbid 22110, Jordan. Tel.: +962 2 7201000x23864; fax: +962 2 7095010.
E-mail address: altarifiaa@vcu.edu (A.A. Altarifi).

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et al., 2013). Different dependent measures are required in preclinical animal studies. For example, we have described "pain-stimulated behaviors" and "pain-depressed behaviors" as two categories of pain-related behavior in animals (Negus et al., 2006; Stevenson et al., 2006). Pain-stimulated behaviors are behaviors that increase in rate, frequency or intensity after delivery of a noxious stimulus, and common examples include tail withdrawal response from noxious thermal stimuli or writhing/stretching responses after intraperitoneal administration of irritants such as dilute acid. Conversely, pain-depressed behaviors are behaviors that decrease in rate, frequency or intensity after delivery of a noxious stimulus, and examples include pain-related reductions in feeding, locomotion, or rates of positively reinforced operant responding. One possibility is that tolerance develops at different rates or to different degrees for different measures of antinociception and/or analgesia.

To address this issue, the primary goal of the present study was to compare the development and expression of morphine tolerance in parallel assays of (1) a pain-stimulated behavior (stimulation of a stretching response), and (2) a pain-depressed behavior [depression of operant responding maintained by electrical brain stimulation in an assay of intracranial self-stimulation (ICSS)], elicited by a common noxious stimulus (intraperitoneal administration of dilute lactic acid) (Negus and Altarifi, 2013; Negus, 2013). We have shown previously that morphine produces dose-dependent and equipotent antinociception in both assays (Pereira Do Carmo et al., 2009; Altarifi et al., 2015). The goal of this study was to assess the degree to which morphine tolerance might also be similar across these two assays.

2. Materials and methods

2.1. Subjects

Twenty-four male Sprague–Dawley rats (Harlan, Frederick, Maryland, USA) weighing 310–350 g at the time of surgery were used. Rats were individually housed and maintained on a 12 h light/dark cycle, with lights on from 06:00 to 18:00 h. Rats had free access to food and water except during testing. Animal maintenance and research were in compliance with National Institutes of Health guidelines on care and use of animals in research, and all animal-use protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

2.2. Assay of intracranial self-stimulation (ICSS)

ICSS has proven to be a useful behavioral assay for preclinical research on expression and treatment of pain-depressed behavior, and the rationale and methods for use of ICSS to study pain-depressed behavior are discussed extensively elsewhere (Negus and Altarifi, 2013; Negus, 2013). For the present study, 13 rats were anesthetized with isoflurane gas (2.5–3% in oxygen; Webster Veterinary, Phoenix, Arizona, USA) for implantation of stainless steel electrodes (Plastics One, Roanoke, Virginia, USA). The cathode of each electrode was 0.25 mm in diameter and covered with polyamide insulation except at the flattened tip, whereas 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 midsaggital 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 subjects were allowed to recover for at least 7 days before commencing ICSS training.

2.2.1. Apparatus

Experiments were conducted in sound-attenuating boxes that contained modular acrylic test chambers ($29.2 \times 30.5 \times 24.1$ 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), stimulus lights (three lights colored red, yellow, and green, positioned 7.6 cm directly above the response lever), a 2 W white house light, and an ICSS stimulator (Med Associates, St. Albans, Vermont, USA). Electrodes were connected to the stimulator with bipolar cables routed through a swivel connector (Model SL2C, Plastics One). The stimulator was controlled by a computer and software that also controlled all the programming parameters and data collection (Med Associates).

2.2.2. Behavioral procedure

After initial shaping of lever-press responding, rats were trained under a fixed-ratio 1 (FR 1) schedule of brain stimulation using procedures similar to those described previously (Altarifi and Negus, 2011; Altarifi et al., 2012, 2013). During experimental sessions, 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. During initial training sessions lasting 30–60 min, the frequency of stimulation was held constant at 158 Hz, and the stimulation intensity for each rat was adjusted gradually to the lowest value that would sustain a high rate of reinforcement (>30 stimulations/min). Once this criterion was met, frequency manipulations were introduced. Sessions involving frequency manipulations consisted of sequential 10-min components. During each component, a descending series of 10 current frequencies (158–56 Hz in 0.05 log increments) was presented, with a 60 s trial at each frequency. A frequency trial was initiated by a 5 s time-out followed by a 5 s "priming" phase, during which subjects received five non-contingent stimulations with a 0.5 s interval between each stimulation. This non-contingent stimulation was then followed by a 50 s "response" phase, during which responding produced electrical stimulation under the FR 1 schedule. Training continued with presentation of up to three sequential components per day, and the current intensity was again adjusted at this stage of training until rats reliably responded for the first three to four frequency trials of all components for at least three consecutive days. This intensity was then held constant for the remainder of the study.

2.2.3. Testing

Once training was completed, tests sessions were initiated. Behavioral tests were conducted and injections administered daily between 12:00 p.m. and 2:00 p.m. Initially, all rats received a single injection of 1.8% lactic acid to confirm sensitivity to acid-induced depression of ICSS prior to further testing. Next, "pre-drug baseline" sessions were conducted over a period of three consecutive days to establish baseline ICSS performance before administration of any dose of morphine. Each pre-drug baseline session consisted of three ICSS components as described above. Rats were then divided into two groups that received either repeated morphine ($N=6$) or repeated vehicle ($N=7$) for seven consecutive days. Rats receiving repeated morphine were treated with 3.2 mg/kg/day on days 1 and 2, 5.6 mg/kg/day on days 3 and 4, and 10 mg/kg/day on days 5, 6, and 7. The control group received daily vehicle (saline) injections. Three ICSS components were conducted before each daily injection, and two additional ICSS components were conducted beginning 30 min after each injection. On days 8, 10, 12, and 14, all animals in both groups were tested with a sequence of four treatments: (1) morphine vehicle+acid vehicle, (2) morphine vehicle+1.8% lactic acid, (3) 1.0 mg/kg morphine+acid vehicle, or (4) 1.0 mg/kg morphine+1.8% lactic acid. Treatment order was counterbalanced