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TOLERANCE, OPIOID-INDUCED ALLODYNIA AND WITHDRAWAL ASSOCIATED ALLODYNIA IN INFANT AND YOUNG RATS

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Abstract—Our laboratory has previously characterized age-dependent changes in nociception upon acute morphine withdrawal. This study characterizes changes in mechanical and thermal nociception following acute, intermittent, or continuous morphine administration in infant (postnatal days 5–8) and young (postnatal days 19–21) rats. Morphine was given as a single acute administration (AM), intermittently twice a day for 3 days (IM), or continuously for 72 h via pump (CM). AM did not produce long-term changes in mechanical or thermal nociception in either infant or young rats. CM produced changes in mechanical nociception that included the development of tolerance, opioid-induced mechanical allodynia and withdrawal-associated mechanical allodynia in young rats, but only tolerance and a prolonged withdrawal-associated mechanical allodynia in infant rats. IM produced withdrawal-associated mechanical allodynia in both infant and young rats. Measuring paw withdrawal responses to thermal stimuli, infant and young rats showed tolerance without opioid-induced thermal hyperalgesia or withdrawal-associated thermal hyperalgesia following CM. In contrast to CM, withdrawal-associated thermal hyperalgesia was seen in both ages following IM. In conclusion, CM versus IM differentially modified mechanical and thermal nociception, suggesting that opioid-dependent thermal hyperalgesia and mechanical allodynia can be dissociated from each other in infant and young rats. Furthermore, tolerance, opioid-induced hypersensitivity, and withdrawal-associated hypersensitivity are age-specific and may be mediated by distinct mechanisms.

Key words: morphine withdrawal, spinal cord, allodynia, hyperalgesia, neonatal rat.

It has long been recognized that morphine exerts two paradoxical actions on the adult nervous system: the inhibition of pain processing manifested as analgesia and the facilitation of nociceptive sensitivity manifested as allodynia and hyperalgesia (Bederson et al., 1990; Kim et al., 1990; Kaplan and Fields, 1991). This facilitation of nociceptive sensitivity can be observed in as little as 20 min post-morphine administration by precipitating withdrawal with an opioid antagonist (Bederson et al., 1990; Kim et al., 1990; Kaplan and Fields, 1991). It has been proposed that allodynia and hyperalgesia upon withdrawal are an unmasking of pronociceptive opioid actions and, hence, are more prominent during withdrawal due to the absence of an opposing analgesic opioid effect (Mao et al., 1994; Vanderah et al., 2001; Mao, 2002). This phenomenon has been termed latent sensitization or latent hyperalgesia (Fry et al., 1980a,b; Basbaum, 1995). Furthermore, the need to escalate opioid dose to maintain analgesia (tolerance) may not be a result of decreased efficacy of opioids but rather reflect the need to overcome increasing opioid-induced facilitation of pronociceptive pathways (Colpaert, 1996; Celerier et al., 1999; Laulin et al., 1999; Vanderah et al., 2000).

The neonatal nervous system is extremely plastic. Dramatic structural and functional reorganization of spinal sensory systems occurs during the neonatal period (Alvares and Fitzgerald, 1999; Fitzgerald and Beggs, 2001). These processes are activity-dependent and thus, abnormal or excessive activity such as that generated by injury may alter normal synaptic development producing changes in somatosensory processing and neurobehavioral sequelae that would not occur in similarly exposed adults (Reynolds and Fitzgerald, 1995; Anand et al., 1999; Ruda et al., 2000). In fact, early exposure to painful stimuli has long-term consequences in both humans and rats (Taddio et al., 1997; Oberlander et al., 2000; Walker et al., 2003). These findings have helped increase the recognition of the need to adequately assess and treat pain in children and infants (Howard et al., 2001). This has led to increasing use of opioids in pediatric patients. Currently, human infants are routinely treated with opioids for pain relief and for the purposes of sedation to permit mechanical ventilation (van Dijk et al., 2002; Simons et al., 2003). With the observation of neonatal abstinence syndrome in 48–84% of infants administered i.v. opioids (Norton, 1988; Arnold et al., 1990; French and Nocera, 1994; Franck and Vilardi, 1995; Franck et al., 1998) it is imperative to assess whether tolerance, opioid-induced pain, and withdrawal pain are present in the pediatric population and whether opioid exposure alters normal synaptic development and produces long-term somatosensory changes (Thornton and Smith, 1998; Thornton et al., 2000).

In previous studies we reported an *in vitro* and *in vivo* model of age-specific acute morphine withdrawal-associated hypersensitivity (Sweitzer et al., 2004a,b). The present study addressed the effect of acute, continuous, and intermittent morphine administration on mechanical thresholds and thermal latencies in postnatal days (P) 5–7

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Abbreviations: AM, acute morphine; CM, continuous morphine; IM, intermittent morphine; P, postnatal day.

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(infant) and P18–21 (young) rats that approximately correspond in neurological developmental to newborn human infants and young children, respectively (Dobbing, 1981). The first part of the study examined mechanical and thermal responses following a single acute administration of morphine (AM). The second part characterized mechanical and thermal responses following repeated intermittent administration of morphine (IM). The third part examined mechanical and thermal responses following continuous infusion of morphine (CM) from an implanted osmotic pump or morphine pellet.

EXPERIMENTAL PROCEDURES

Animals

P5–12 (infant) and P18–24 (young) Sprague–Dawley rats (Charles River Laboratories, Raleigh, NC, USA) of both sexes were housed in litters culled at 10 pups/dam in a 12-h light/dark cycle (lights on at 7 a.m.) with food and water available *ad libitum*. All experimental protocols were approved by the Institutional Animal Care and Use Committees at Stanford University and the University of South Carolina. All experiments conformed to the guidelines of the Committee for Research and Ethical issues from the International Association for the Study of Pain (1983). Efforts were made throughout the experiment to minimize animal discomfort and to reduce the number of animals used. For all behavioral experiments rats were maintained at nesting temperature with overhead heat lamps whenever the pups were separated from the dams. Animal weights were measured daily.

Maternal care

There are numerous reports that maternal care can alter offspring behavior (Barron and Riley, 1985; Fleming et al., 1999; Champagne and Meaney, 2001; Huot et al., 2001; Meaney, 2001). There is evidence that experimental perturbation of a litter leads to the whole litter receiving similar disrupted care and that maternal care is driven by the pups and not by the dams making it difficult to control for individual pup–dam interactions (Huot et al., 2001; Marino et al., 2002). We can control for overall maternal care at the litter level (for example how much time the dam spends in different nursing postures). For this study, treatments were randomized within each litter so that pups from each treatment were exposed to the same dam. Thus, multiple dams are represented within each treatment group to control for the effect of maternally driven disrupted care on offspring behaviors (Huot et al., 2001; Marino et al., 2002). This design does not preclude pup-driven differential treatment of individual pups by the dam (e.g. amount of anal–genital licking for each pup).

Experimental design: AM

Following measurement of baseline mechanical threshold and thermal latency a single s.c. injection of morphine (0.5, 1, 3, 6, or 12 mg/kg) or saline was given on P7 or P20. Mechanical threshold and thermal latency were measured at 15 and 30 min post-morphine, respectively and then daily for 4 days. The experimental design is shown schematically in Fig. 1A.

Repeated IM

In P5–7 and P18–21 rats, morphine (0.5, 1, or 3 mg/kg for a total daily dose of 1, 2, or 6 mg/kg/day) or saline (s.c. in 50 μl volume) was administered twice a day for 3 days (10 a.m. and 5 p.m.) with a final administration on day 4 (10 a.m.). Mechanical threshold and thermal latency were measured immediately prior to the first morphine exposure each morning as well as at 1 h post-exposure. On day 4 mechanical threshold and thermal latency were measured prior to morphine or saline administration as well as at 1 and 4 h post-morphine or saline and then daily to P11 or P24. The experimental design is shown schematically in Fig. 1B.

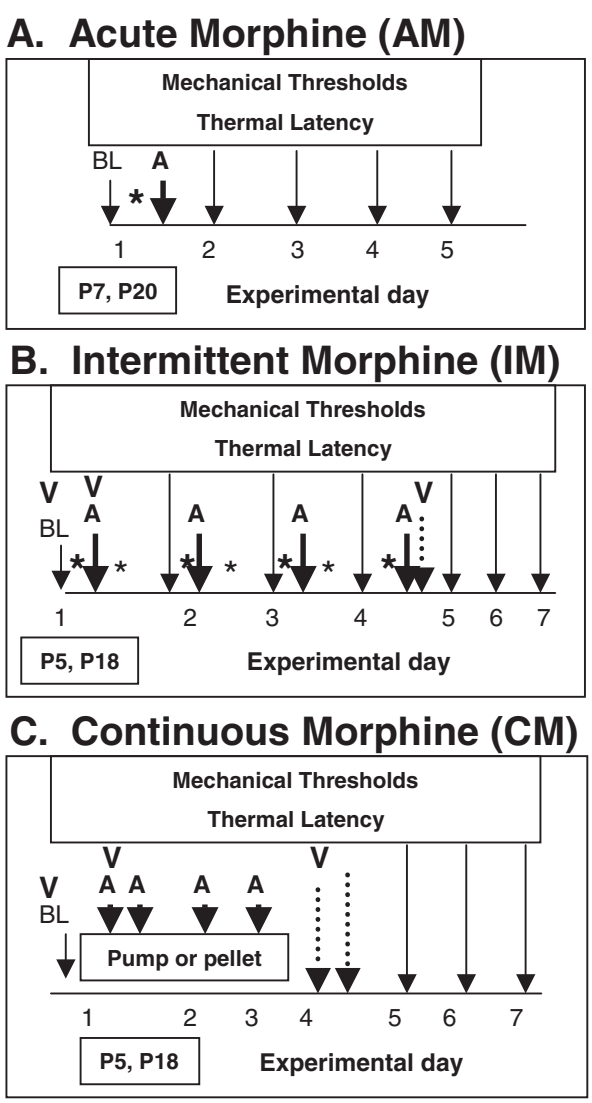

Fig. 1. Experimental design for acute morphine administration (A), repeated intermittent morphine (B), and continuous morphine exposure using an s.c. mini osmotic pump or morphine pellet (C) in P5–P7 or P18–P20 rats. BL represents collection of baseline mechanical and thermal responses and baseline activity measures. Morphine administration is indicated by an asterisk (*). For acute morphine experiments this occurred once on P7 or 20. Intermittent morphine studies began on P5 or 18 with administration twice a day for 3 days followed by a single administration on experimental day 4. Morphine pumps or pellets were implanted on P5 or 18 for continuous morphine delivery for 72 h. The A arrow represents time points at which post-morphine analgesia (mechanical and thermal) was assessed. The long arrows indicate mechanical and thermal measures during the withdrawal and abstinence periods. The dashed arrows indicate time points at which acute withdrawal was being assessed. The time points at which animals were videotaped are indicated by a V.

CM: osmotic pump

Baseline mechanical threshold and thermal latency were collected on P5 or P18. Mini pumps were implanted as previously described