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# Differential ontogenesis of thermal and mechanical antinociception induced by morphine and β-endorphin

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**Abstract**

The antinociceptive effects induced by β-endorphin and morphine given supraspinally have been previously demonstrated to be mediated by the activation of different neural mechanisms. The present experiments were to examine the effects of intraventricular administration of β-endorphin and morphine in mechanical paw-withdrawal and thermal tail-flick nociceptive tests in rats of 2–28 days of age. 2–4-day-old neonates were not responsive to i.c.v. injection of β-endorphin or morphine for the inhibition of the tail-flick response. The thermal antinociceptive responses induced by β-endorphin and morphine started to develop in 7–14-day-old rats and continued to increase at 21–28 days. The inhibition of the mechanical paw-withdrawal response to β-endorphin was already present in 2-day-old rats and morphine in 4-day-old rats. The mechanical antinociception progressively increased and reached a plateau at 7 days of age for β-endorphin and 28 days of age for morphine. β-Endorphin was found to be more efficacious than morphine in producing mechanical antinociception. The results demonstrate that β-endorphin- and morphine-induced antinociception to mechanical and thermal stimuli develops differently and are consistent with the hypothesis that two descending pain inhibitory systems activated by β-endorphin and morphine are differentially developed.

*Keywords:* β-Endorphin; Morphine; Ontogenesis; Antinociception

## 1. Introduction

In a series of experiments, it has been established that the antinociceptive effects induced by morphine and β-endorphin are mediated by the activation of different neural mechanisms. β-Endorphin-induced antinociception measured by the tail-flick response is mediated by the stimulation of ε-opioid receptors at supraspinal sites while morphine-induced antinociception is mediated by the stimulation of supraspinal μ-opioid receptors. The stimulation of the ε-opioid receptors by β-endorphin results in the spinal release of [Met$^5$]enkephalin and subsequent stimulation of δ-opioid receptors in the spinal cord for antinociception while the activation of μ-opioid receptors by morphine induces the spinal release of norepinephrine and serotonin and stimulation of $\alpha_2$-adrenoceptors and 5-HT receptors for antinociception (Tseng and Fujimoto, 1984, 1985; Suh and Tseng, 1988, 1990; Suh et al., 1989; Tseng and Tang, 1989, 1990).

Many studies have demonstrated that opioids can produce antinociception in neonate animals including human infants (see McDowell and Kitchen, 1987 for review; Yaster, 1987). Studies of antinociception at different ages have found that rat and mouse neonates become more responsive to opioid-induced antinociception as they mature (Alleva and Laviola, 1987; Barr et al., 1986; Giordano and Barr, 1987; Johannesson and Becker, 1973; Pasternak et al., 1980; Zhang and Pasternak, 1981). The onset of antinociception can be quite abrupt (Barr et al., 1986; Spear et al., 1985). The developmental course of antinociception differs from opioids that prefer different receptors (Barr et al., 1986; Giordano and Barr, 1987; Helmstetter et al., 1988; Pasternak et al., 1980; Zhang and Pasternak, 1981) in part because different receptor types act on different neuroanatomical sites that mature differently.

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Previous studies by others of the ontogeny of the opioid-induced antinociception have focused mainly on opioid $\mu$- and $\kappa$-opioid receptor agonists (Barr et al., 1986, 1992; Giordano and Barr, 1987; Allerton et al., 1989). The ontogeny of opioid $\epsilon$-receptor agonist, $\beta$-endorphin, on the production of antinociception has not been studied. The present study was thus designed to compare the ontogeny of morphine- and $\beta$-endorphin-induced antinociception measured by thermal tail-flick and mechanical paw-withdrawal responses.

## 2. Materials and methods

### 2.1. Animals

The pregnant female Sprague-Dawley rats (Sasco Inc., Omaha, NE) were delivered to the animal facilities and were housed individually in plastic cages with food and water ad libitum in a room maintained at $22 \pm 0.5$°C with an alternating 12 h light-dark cycle. The neonates of either sex, at ages of 2, 4, 7, 14, 21 and 28 days old were used for the studies. Animals were separated from their dams, weighed, and marked with a marking pen for identification. Members of each litter were randomly assigned to treatment conditions and retained in groups in small plastic tubs with pine chip bedding.

### 2.2. Drug administration in different ages of neonatal rats

For i.c.v. injection of morphine or $\beta$-endorphin, neonates at different ages were lightly anesthetized with halothane and an incision was made to expose bregma. Rats were injected i.c.v. under halothane anesthesia according to the procedure described by Haley and McCormick (1957) for i.c.v. injection in mice. The injection volume was 4 $\mu$l. The injection site was 1 mm lateral to bregma. The depth of the injection from the surface of the skull was 3.5 mm for 2–7-day-old rats, 4.0 mm for 14–21-day-old rats and 4.5–5 mm for 28-day-old rats. The rats recovered from halothane anesthesia in 1 to 2 min after injection.

### 2.3. Antinociceptive tests

The antinociceptive responses to morphine or $\beta$-endorphin were determined by the thermal tail-flick response (D'Amour and Smith, 1941) and mechanical paw-withdrawal response (Randall and Selitto, 1957). For measurement of the latency of the tail-flick response, neonates were gently held by hand with their tail positioned in the apparatus (EMDIE Instrument Co., Maidens, VA, Model TF6) for radiant heat stimulation on the dorsal surface of the tail. The intensity of the heat stimulus was initially adjusted for 2-day-old neonates in which the animals flicked their tails in 3–5 s. This setting was fixed throughout the experiments in order to compare the sensitivity to thermal stimulation in different ages of neonates. The mechanical nociceptive response was determined by using a modification of the Randall-Selitto test. In this test, the hind paw was placed between the stylus and platform of an Ugo-Basile analgesiometer and pressure to the paw increased at a fixed rate. The pressure, in grams, at which the animal struggled to remove its paw was termed the paw withdrawal threshold. The inhibition of the tail-flick and paw-withdrawal responses was expressed as 'percent maximum possible effect (% MPE)' which was calculated as: $[(T_1-T_0)/(T_2-T_0)] \times 100$, where $T_0$ and $T_1$ were the tail-flick latency or the weight for paw-withdrawal before and after the injection of opioid receptor agonist and $T_2$ was the cutoff time which was set at 10 s and 25 g for tail-flick and paw-withdrawal tests, respectively.

## 3. Results

### 3.1. Changes of the sensitivities to the tail-flick and paw-withdrawal responses in neonatal rats

Fig. 1 shows the changes of the sensitivities to radiant heat stimulation for the tail-flick response and to mechanical pressure for the paw-withdrawal response in different ages of neonatal rats. Neonates, 2–7 days old, were relatively more sensitive to thermal and mechanical stimulation and had shorter latencies for the tail-flick and paw-withdrawal responses. The thresholds of latencies of both tests increased from ages 3–14 days, reached a plateau at 14 days, then remained steady in older rats.

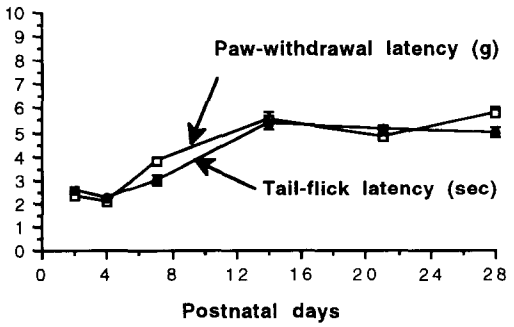


Fig. 1. Changes in nociceptive latencies of neonate rats from 2 to 28 days. The latency of paw withdrawal after pressure stimuli (g) and the latency of tail-flick withdrawal after thermal stimuli (s) were measured as described in the text. Vertical bars indicate the S.E.M. $n = 8$–10 rats for each point.

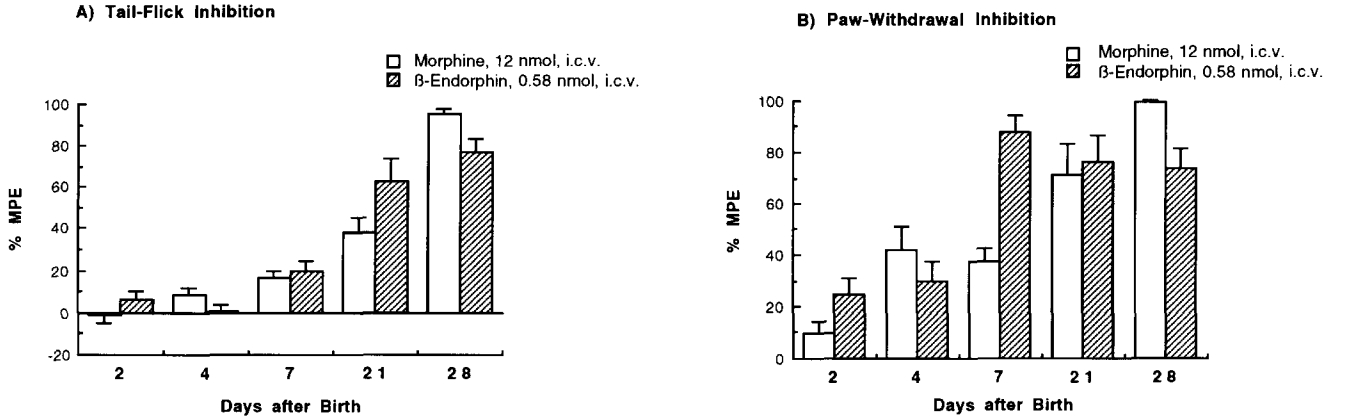


Fig. 2. The development of the inhibition of tail-flick responses (A) and paw-withdrawal responses (B) induced by i.c.v. administered morphine (12 nmol, open bars) and β-endorphin (0.58 nmol, hatched bars) in 2-, 4-, 7-, 21- and 28 day-old rats. The response to nociceptive stimuli was measured 20 and 30 min after i.c.v. injection of morphine and β-endorphin, respectively. Vertical bars indicate the S.E.M. $n = 8$ rats for each group.

### *3.2. Inhibition of the tail-flick and paw-withdrawal response induced by i.c.v. administration of β-endorphin or morphine in different ages of neonates*

Previous studies have reported that β-endorphin given supraspinally is about 20 fold more potent than morphine in producing antinociception in adult rats (Tseng and Tang, 1990; Tseng and Wang, 1992). 2 μg (0.58 nmol) of β-endorphin and 4 μg (12 nmol) of morphine sulfate were then chosen for the study because these doses of β-endorphin and morphine produce about equi-antinociceptive effects in adult rats. 2–4-day-old neonates were not responsive to i.c.v. injection of β-endorphin or morphine for the inhibition of the tail-flick response. The inhibition of the tail-flick response induced by β-endorphin and morphine given i.c.v. started to develop in 7–14-day-old rats and continued to increase in 21–28-day-old rats (Fig. 2a). Unlike the inhibition of the tail-flick response induced by β-endorphin and morphine, the inhibition of the mechanical paw-withdrawal response induced by β-endorphin given i.c.v. was already present in 2-day-old rats and by morphine in 4-day-old rats. The inhibitory effects progressively increased, reached a plateau at 7 days for β-endorphin and at 21 days for morphine (Fig. 2b).

Fig. 3 compares the dose-response effects of β-endorphin and morphine given i.c.v. on the inhibition of the tail-flick and paw-withdrawal responses in 14-, 21- and 28 day-old rats. β-Endorphin given i.c.v. was found to produce full sensitivity as expressed in dose-response curves for β-endorphin-induced tail-flick inhibition in 14 day-old rats. The sensitivity to β-endorphin was only increased slightly in 21- and 28-day-old rats. β-Endorphin given i.c.v. also produced full sensitivity in inhibiting mechanical paw-withdrawal response in 14-day-old rats. The sensitivity to β-endorphin for the inhibition of the paw-withdrawal response was attenu-

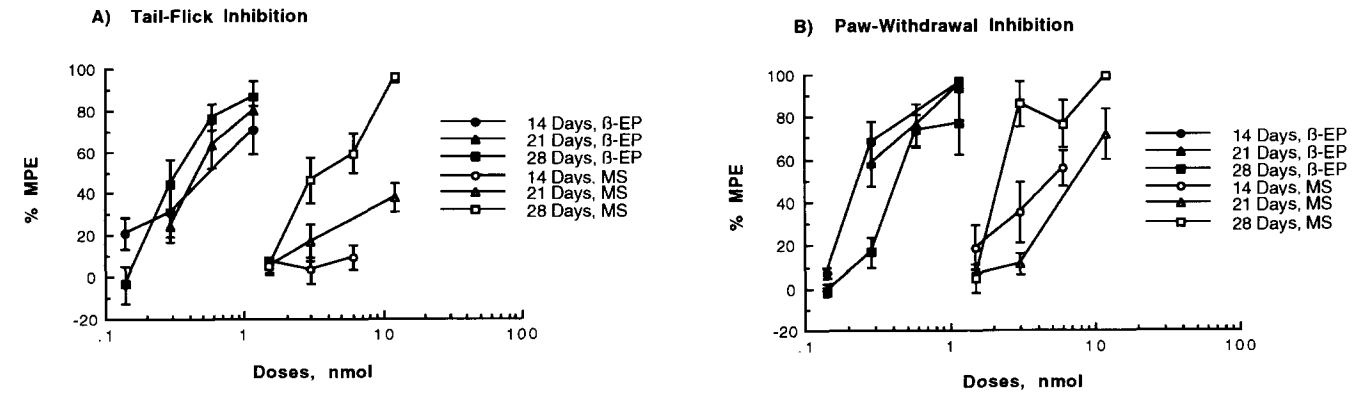


Fig. 3. The effect of varying doses of i.c.v. administered morphine or β-endorphin on the inhibition of the tail-flick response (A) and paw-withdrawal response (B) in 14-, 21- and 28-day-old rats. The response to nociceptive stimuli was measured 20 and 30 min after i.c.v. injection of morphine (1.2–12 nmol) and β-endorphin (0.12–1.2 nmol), respectively. Vertical bars indicate the S.E.M. $n = 8$ rats for each group.

ated slightly in 28-day-old rats as evidenced by the finding that dose-response curves were shifted to the right. On the other hand, morphine given i.c.v. was found to be much less sensitive in inhibiting the tail-flick and paw-withdrawal responses in 14-day-old compared with that in 21- and 28-day-old rats. The sensitivity of rats to morphine to both nociceptive tests were progressively increased in 21- and 28-day-old rats; the dose-response curves of morphine for the tail-flick and paw-withdrawal inhibition were shifted progressively to the left as the rats got older.

## 4. Discussion

It has been proposed that different neurotransmitters are used for thermal and mechanical noxious stimulation in the primary afferent fibers. Kuraishi et al. (Kuraishi et al., 1985b; Ohno et al., 1988) proposed that somatostatin and substance P transmit the information of thermal and mechanical nociception in the dorsal horn of the spinal cord, respectively. Both somatostatin and substance P fibers which are concentrated in the dorsal horn of the spinal cord are present at the time of the birth (Marti et al., 1987; Hammond and Ruda, 1991). Marti et al (1987) observed that a pronounced increase in the number of somatostatin and substance P fibers in the substantia gelatinosa occurs perinatally and the peptides continue to increase after the birth. In the present study, age-dependent alteration in both thermal and mechanical nociceptive thresholds were observed. We found that 2–4-day-old neonates have already developed high sensitivity to both thermal and mechanical nociceptive stimulation. The thresholds to thermal and mechanical nociception increased gradually, reached a plateau at 14 days and then remained steady. Thus neurotransmitter systems for the transmission of thermal and mechanical nociceptive transmission appears to be well developed at the time of the birth of neonate rats and they may develop at different courses as they mature.

The present study demonstrated the differential development of $\beta$-endorphin and morphine on the inhibition of the mechanical pressure-induced paw-withdrawal and radiant heat-induced tail-flick responses. The neonates appear to develop the antinociceptive response to $\beta$-endorphin and morphine induced by mechanical nociceptive stimulation earlier than that induced by thermal nociception. The inhibition of the mechanical paw-withdrawal responses produced by $\beta$-endorphin and morphine developed relatively early with 2–4-day-old neonates demonstrating modest antinociceptive responses. The inhibition developed progressively and reached maximal in 7-day-old neonates for $\beta$-endorphin and in 28-day-old rats for morphine. The inhibition of the thermal tail-flick responses induced by $\beta$-endorphin and morphine appeared later with low antinociceptive behavior at 7 days. The inhibition developed progressively and reached maximal in 28-day-old neonates. Our results of the experiment of morphine-induced antinociception were consistent with the report by Barr et al. (1992). They reported that morphine given i.c.v. produced antinociception induced by mechanical but not thermal nociceptive stimuli in 10-day-old rat neonates.

The exact mechanisms underlying the differential ontogenesis of $\beta$-endorphin and morphine on the inhibition of the mechanical paw-withdrawal and the thermal tail-flick response are not clear at this time. The mechanical stimulus was applied to the hindpaw and the thermal tail-flick stimulus to the tail. The differences that we found could be attributable to either the stimulus differences or the location differences. However, Giordano and Barr (1987) and Blass et al. (1993) reported that opioid-induced antinociception in the tail develops prior to that of the hind paw, excluding the possibility that the earlier development of the hindpaw mechanical analgesia is related to the differences of the location of stimulation.

Alternatively, the differential development of the descending pain controls activated by $\beta$-endorphin and morphine for inhibiting these two nociceptive responses should be considered. The antinociception induced by morphine applied supraspinally has been demonstrated to be mediated by the activation of descending serotonergic and noradrenergic systems. Barr and Kuraishi et al. (Giordano and Barr, 1988; Hughes and Barr, 1988; Kuraishi et al., 1983, 1985a) proposed that descending serotonergic and noradrenergic systems are important for morphine induced antinociception evoked by thermal and mechanical nociceptive stimuli, respectively. Depletion of serotonin by intrathecal injection of 5,7-dihydroxytryptamine attenuated morphine antinociception when the thermal stimulus was applied in neonate rats (Giordano and Barr, 1987). The antinociceptive action of morphine was only slightly attenuated when mechanical stimulus was applied. The antinociception induced by intrathecal injection of norepinephrine or clonidine were more pronounced against a mechanical than thermal stimuli observed in 10-day-old neonates (Hughes and Barr, 1988). Therefore it is possible that differential development of the neurotransmitter systems may account for the differential effects of morphine on the production of antinociception evoked by thermal and mechanical nociceptive stimuli.

$\beta$-Endorphin was found to be more efficacious than morphine in inhibiting the mechanical paw-withdrawal response. $\beta$-Endorphin at 0.58 nmol given i.c.v. produced an age-dependent inhibition of the paw-withdrawal response in 2–7-day-old neonates and the antinociception reached a plateau in 7-day-old neo-

nates, while morphine at 12 nmol given i.c.v. produced an age-dependent inhibition observed in 4–28-day-old neonates. The dose-response studies of β-endorphin and morphine on the inhibition of the mechanical paw-withdrawal response in 14–28-day-old rats also indicate that neonate rats developed earlier in antinociceptive response to β-endorphin than morphine. β-Endorphin given i.c.v. produced full sensitivity in inhibiting mechanical paw-withdrawal responses in 14-day-old rats, while morphine given i.c.v. was found to be much less sensitive in inhibiting paw-withdrawal responses in 14-day-old compared with responses of 21- and 28-day-old rats.

The antinociception induced by β-endorphin has been previously demonstrated to be mediated by the stimulation of ε-opioid receptors and subsequently release of [Met$^5$]enkephalin acting on δ-opioid receptors in the spinal cord. At birth, enkephalin terminal field in the spinal cord and in the brain regions have achieved their mature distribution, increasing only in intensity throughout postnatal development (Senba et al., 1982; Palmer et al., 1982; Romagnano et al., 1989; Loughlin et al., 1985). In contrast to μ- and κ-opioid receptor sites which significant density of receptors sites in the spinal cord and the brain are apparent at birth, the developmental appearance of δ-opioid receptor is largely postnatal. Although some δ sites are detectable in the brain and spinal cord, there is a major increase in receptor expression after the first postnatal weeks (Kornblum et al., 1987; Petrillo et al., 1987; Spain et al., 1985). The ontogenetic developments of the [Met$^5$]enkephalin and δ-opioid receptor correlates well with the ontogenetic development of β-endorphin-induced antinociception.

The antinociception induced by morphine given supraspinally is mediated by the stimulation of μ-opioid receptors and subsequent activation of descending noradrenergic and serotonergic systems in the spinal cord. Therefore the development of the descending serotonergic and noradrenergic fibers is an important consideration. The first serotonergic axons are observed in the white matter at 18 prenatal days and have entered the gray matter at birth, although are still very sparse and diffuse at this stage (Bregman, 1987). An adult pattern of serotonergic innervation in the spinal cord is not achieved until 14 postnatal days in the cervical cord and 21 postnatal days in the lumbar cord. Noradrenergic containing terminals appear earlier in the dorsal horn than in serotonin at about 4 postnatal days (Commissiong, 1983; Herregodts et al., 1990; Loizou, 1972; Marti et al., 1987) and levels are mature by approximately 2 postnatal weeks. The ontogenetic development of serotonergic and noradrenergic innervation appear to be correlated well with the ontogenetic development of morphine antinociception. It appears that the development of the ε system which involves [Met$^5$]enkephalin and δ-receptor is developed earlier than μ system which involves noradrenergic and serotonergic system.

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