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Long-term alterations in opiate antinociception resulting from infant fentanyl tolerance and dependence

Suzanne R. Thornton [a], Forrest L. Smith [b,*]

[a] *Department of Pharmacology, UMDNJ, Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635, USA*
[b] *Department of Pharmacology and Toxicology, Medical College of Virginia Campus of Virginia Commonwealth University, P.O. Box 980613, Richmond, VA 23298-0613, USA*

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Abstract

Postnatal day-14 (P14) infant rats remained naive or were implanted with osmotic minipumps infusing saline or fentanyl (50 μg kg^{-1} h^{-1}). Fentanyl was administered 72 h later for measurement of antinociception in the tail-flick test. The potency of fentanyl was 3.0-fold lower in fentanyl-infused compared to saline-infused P17 rats. Fentanyl-infused P17 rats injected with naloxone underwent withdrawal characterized by increases in spontaneous activity, wall climbing, diarrhea, abdominal stretching, forepaw treading/tremors, wet-dog shakes, jumping, ptosis, rhinorrhea and hypothermia. Other naive, saline-infused and fentanyl-infused P17 rats not challenged with fentanyl or naloxone were housed until maturing into P42 juveniles. Fentanyl's potency was equal among each treatment group. However, morphine's potency was reduced in juveniles tolerant to fentanyl as infants. Morphine was also less potent in P90 adults tolerant to fentanyl as infants. Thus, chronic opiate exposure during infancy may affect the developing central nervous system, and desensitize animals and humans to opiate analgesia throughout life.

Keywords: Tolerance, neonatal; Fentanyl; Physical dependence; Alzet osmotic minipump

1. Introduction

Reports indicate that physicians are now more likely to use opiates to manage pain in neonates and infants than in the past (Purcell-Jones et al., 1987; Yaster, 1987; Sukhani, 1989; McLaughlin et al., 1993). Opiates are routinely administered i.v. to provide continuous analgesia and sedation during extracorporeal membrane oxygenation and mechanical ventilation, for the treatment of life-threatening pulmonary diseases in neonates and infants (Arnold et al., 1990, 1991; Roth et al., 1991; Leuschen et al., 1993). Considerable evidence indicates that iatrogenic tolerance and dependence can develop in this population receiving fentanyl or morphine by continuous intravenous administration (Maguire and Maloney, 1988; Norton, 1988; Arnold et al., 1990, 1991; Noerr, 1991; French and Nocera, 1994; Katz et al., 1994; Franck and Vilardi, 1995). Iatrogenic tolerance was indicated when a given dose of fentanyl or morphine became ineffective and the patient required increasingly larger doses to provide the same level of analgesia observed with smaller doses. Physical dependence to morphine and fentanyl was characterized by the presence of withdrawal signs when the drug was discontinued. Fifty to 84% of neonates removed from fentanyl exhibited abstinence within a 24-h period and 48% exhibited signs with morphine withdrawal (Norton, 1988; Arnold et al., 1990, 1991; French and Nocera, 1994). To our knowledge, nothing is known about the long-term consequences of iatrogenic tolerance and dependence in neonates and infants as they mature into juveniles and adults.

In animals, the long-term consequences of in utero morphine, methadone and heroin exposure have been examined. In utero opiate exposure alters opioid receptor density (Kirby and Aronstam, 1983; Tempel et al., 1988; Hammer et al., 1991; Tempel, 1991), and neural development (Smith et al., 1977; Zagon and McLaughlin, 1977a,b; Hammer and Hauser, 1992). Behavioral consequences have also been observed. These animals exhibit an altered sensitivity to opiate antinociception, which is described in detail in Section 4.2 (O'Callaghan and Holtzman, 1976; Zagon and McLaughlin, 1981; Kirby et al., 1982; Enters et al.,

* Corresponding author. Tel.: +1-804-828-5596 (office), +1-804-828-8446 (lab); Fax: +1-804-828-2117; E-mail: flsmith@hsc.vcu.edu

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1991). Rats that were exposed in utero to morphine or methadone self-administer greater amounts of heroin or morphine than control offspring (Glick et al., 1977; Hovious and Peters, 1985; Ramsey et al., 1993). Other long-term consequences include delays in the negative geotaxis and vertical screen tests, as well as increases in spontaneous activity, and more learning disabilities (Sobrian, 1977; Peters, 1978; Zagon et al., 1979; Zagon and McLaughlin, 1984; Enters et al., 1991).

We have developed a model of neonatal rat fentanyl tolerance and dependence that simulates the postnatal exposure of human neonates to opiates (Thornton and Smith, 1995). The model was developed to not only characterize tolerance and dependence, but to also examine the long-term consequences in these animals later in life. For these studies, infant postnatal day-14 (P14) rats were rendered tolerant and dependent to fentanyl to test the hypothesis that these animals as juveniles and adults would exhibit a decreased sensitivity to opiate antinociception. Our results indicate that human neonates and infants later in life might require larger doses of some opiates to manage pain.

2. Methods

2.1. Source of infant rats

Nulliparous female Sprague–Dawley dams and appropriately aged litters of 10 pups (five females and five males) were purchased from Zivic-Miller (Zedianople, PA). The animals were housed in the animal care facilities at the Medical College of Virginia with a 12-h light–dark cycle, and allowed food and water ad libitum. The animals arrived in the facility at age P8 and remained there until P14. At P14, the infants were surgically implanted with osmotic minipumps, and remained with the dam before conducting the experiments 72 h later. Experiments were conducted with approval of the Institutional Care and Use Committee at the Medical College of Virginia.

2.2. Surgical implantation of osmotic minipumps

Alzet osmotic minipumps were surgically implanted as previously described in detail (Thornton and Smith, 1995; Thornton et al., 1997). Briefly, P14 infant rats were briefly anesthetized with methoxyflurane for implantation of Alzet 1003D osmotic minipumps. Within each litter of five female and five male rats, two were anesthetized but remained naive, while eight were randomly assigned to receive saline- or fentanyl-filled pumps. Fentanyl concentrations were adjusted to deliver at a rate of 50 $\mu g\ kg^{-1}\ h^{-1}$. Seventy-two hours later, the P17 rats were injected with fentanyl s.c. to assess for antinociception, or naloxone s.c. to assess for physical dependence. Other animals not injected with fentanyl or naloxone on P17 were assessed for antinociception as juveniles and adults.

2.3. Tail-flick test

The tail-flick test used to assess antinociception was developed by D'Amour and Smith (1941) and modified by Dewey et al. (1970). Before s.c. injection of drug, base-line (control) tail-flick latencies were measured for each animal. The heat stimulus was adjusted to yield base-line latencies of 3 to 4 s, and a 10 s cut-off was used to prevent tissue damage (Enters et al., 1991; McLaughlin and Dewey, 1994; Thornton and Smith, 1995). Peak fentanyl antinociception at 10 min was based on two previous studies in this laboratory (Thornton and Smith, 1995; Thornton et al., 1998). At peak antinociception, the test latency was measured and the data were transformed to the percentage of maximum possible effect (%MPE) according to the method of Harris and Pierson (1964). This was calculated as: $\%MPE = [(\text{test latency} - \text{control latency})/(10 - \text{control latency})] \times 100$. The ED_{50} value and 95% confidence limits for dose–response curves were calculated using the method of Bliss (1967). Potency ratios and 95% confidence limits (C.L.) were calculated using the method of Colquhoun (1971), where a potency ratio of greater than one, and the lower 95% C.L. greater than one, is considered significant.

2.4. Withdrawal testing

Naloxone (5 mg kg^{-1}, s.c.) was administered to P17 naive rats or rats chronically infused with saline or fentanyl (50 $\mu g\ kg^{-1}\ h^{-1}$) for 72 h. Animals from each treatment group were randomly selected from among the litters during the study. Following naloxone administration, the animal was moved to a Plexiglas cage for a 15 min observation period. A cage measuring 50×31 cm was marked with a grid of 30 squares (8×7 cm) for measurement of spontaneous activity. The average number of lines crossed in 15 min was expressed as lines/animal. Other behaviors were quantified as the number of animals exhibiting the sign/total number of animals observed.

2.5. Surgical removal of Alzet osmotic minipumps

For studies on the long-term consequences of infant fentanyl exposure, osmotic minipumps were removed on P19. Opiate infusion from the Alzet 1003D pump is guaranteed for 72 h. Thus, the animals were infused with saline or fentanyl for 72 h from P14 to P17. The pump remained in the animals two additional days to allow for a slow withdrawal from the fentanyl supplied by the pump. On P19, the rats were briefly anesthetized with methoxyflurane and placed on a 37°C heating pad. Fur was depilated from the previous implantation site, and the area was swabbed with 70% ethanol. The initial incision was opened with sterile scissors, and the osmotic minipump was removed with sterile forceps. The subcutaneous space that housed the osmotic minipump was closed with Vetbond

Tissue Adhesive (3M Animal Care Products, St. Paul, MN), and swabbed with 10% providone iodine (General Medical, Prichard, WV). The animals received 60,000 U of potassium penicillin G i.p. to prevent infection. The animals were ear-tagged for identification and returned to the dam. Forty-eight hours later, the P21 animals were weaned and housed in groups of two per cage until P42, when they were challenged with fentanyl s.c. At P55 and P90, the same animals were again challenged with morphine s.c.

2.6. Drugs and solutions

Crystalline fentanyl hydrochloride (National Institute on Drug Abuse, Bethesda, MD), morphine Sulfate (Research Biochemicals International, Natick, MA) and naloxone hydrochloride (Sigma, St. Louis, MO) were dissolved in sterile pyrogen-free isotonic saline (Baxter Healthcare, Deerfield, IL). Potassium penicillin G (various suppliers) was diluted and injected i.p. to prevent infection.

3. Results

3.1. Fentanyl tolerance in infant rats

Experiments were conducted to test the hypothesis that continuous fentanyl administration renders infant rats tolerant to fentanyl. For these experiments, P14 rats remained naive or were infused with saline (1 μl h^{-1}) or fentanyl (50 μg kg^{-1} h^{-1}), and were tested for tolerance 72 h later. Baseline tail-flick latencies following the infusion were not different among the groups. However, the potency of acutely administered fentanyl was significantly reduced in the fentanyl-infused rats (Fig. 1). Not only were the ED_{50} values increased (Table 1), but fentanyl's potency was decreased 3.0-fold. It is noteworthy that the saline-pump implanted rats exhibited similar ED_{50} values as naive animals, which indicated the absence of a pump effect on antinociception.

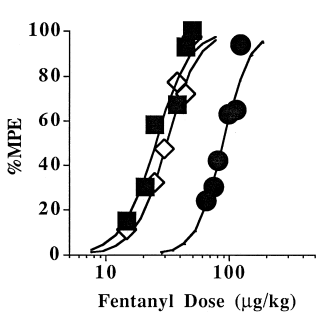

Fig. 1. Tolerance to the antinociceptive effects of fentanyl in P17 rats. P14 rats remained naive or were surgically implanted with 1003D Alzet osmotic minipumps. Seventy-two hours after infusion of saline (1 μl h^{-1}) or fentanyl (50 μg kg^{-1} h^{-1}), baseline tail-flick latencies were obtained from P17 rats. Ten minutes after injecting fentanyl, antinociception was measured in naive (■), saline- (◇, 1 μl h^{-1}) or fentanyl-infused (●, 50 μg kg^{-1} h^{-1}) rats. Each dose–response curve represents 20 to 25 rats.

Table 1
Tolerance to fentanyl in P17 rats after a 72 h infusion of fentanyl (50 μg kg^{-1} h^{-1}) from osmotic minipumps

Treatment	ED_{50} (C.L.) μg kg^{-1}	Potency ratio (C.L.)
Naive	25.7 (21.6 to 29.7)	–
Saline pump		vs. Naive
(1 μl h^{-1})	31.6 (27.2 to 40.0)	1.13 (0.96 to 1.34)
Fentanyl pump		vs. Saline-P
(50 μg kg^{-1} h^{-1})	90.1 (80.6 to 99.5)[a]	3.01 (2.60 to 3.50)[a]

[a]Significantly different from saline pump animals.
P14 rats remained naive or were implanted with an osmotic minipump containing saline or fentanyl. Seventy-two hours later, antinociception in P17 rats was measured the tail-flick test 10 min after s.c. administration of fentanyl.

3.2. Physical dependence to fentanyl in infant rats

The hypothesis was also tested that continuous fentanyl administration renders P17 infant rats physically dependent to fentanyl. For these experiments, P14 rats remained naive or were infused with saline or fentanyl for 72 h. Fentanyl-infused P17 rats administered naloxone (5 mg kg^{-1}, s.c.) displayed a precipitated withdrawal syndrome (Table 2). Fentanyl-infused rats displayed the highest level of spontaneous activity, which often resulted in wall climbing behavior. Although many naive and saline-infused rats

Table 2
Behavioral profile of fentanyl withdrawal precipitated by 5 mg kg^{-1} naloxone in P17 rats

Withdrawal signs	Naive	Saline pump	Fentanyl pump
Spontaneous activity (lines/animal)	156	203	334[a]
Micturation	5/9	8/9	9/9
Defecation	6/9	8/9	9/9[c]
Face washing	9/9	9/9	4/9
Wall climbing	0/9	0/9	5/9[b]
Abdominal stretching	0/9	0/9	7/9[b]
Forepaw treading/tremors	0/9	0/9	9/9[b]
Scream on touch	0/9	0/9	5/9[b]
Wet-dog shakes	0/9	0/9	6/9[b]
Spontaneous jumping	0/9	0/9	6/9[b]
Mastication	7/9	6/9	8/9
Ptosis	0/9	0/9	9/9[b]
Rhinorrhea	0/9	0/9	9/9[b]
ΔT_b (°C)	−0.06	−0.42	−1.8[a]

[a]Significantly different from respective saline pump-implanted rats, ANOVA with post hoc Tukey's test.
[b]Significantly different from respective saline pump-implanted rats, χ^2.
[c]Diarrhea.
Rats at P14 remained either naive or were infused with saline (1 μl h^{-1}) or fentanyl (50 μg kg^{-1} h^{-1}) from osmotic minipumps. At P17, the rats injected naloxone were observed for 15 min for signs of dependence. These behaviors are indicated as the number of animals exhibiting the sign to the total number of animals observed.

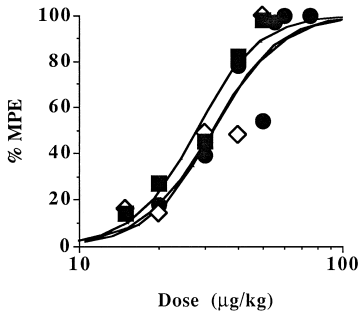

Fig. 2. Rats tolerant to fentanyl as infants are equally sensitive as opioid-naive animals to fentanyl antinociception as juvenile P42 rats. P14 rats remained naive (■) or were infused with saline (◇, 1 μl h^{-1}) or fentanyl (●, 50 μg kg^{-1} h^{-1}) for 72 h. Five days later, after removal of the minipumps, the rats were housed in groups of two until they matured into juveniles. Antinociception was measured in P42 juvenile rats 10 min after s.c. administration of fentanyl. Each dose–response curve represents 20 to 25 rats.

defecated, only the fentanyl-infused rats exhibited diarrhea. The fentanyl-infused rats exhibited abdominal stretching similar to the visceral nociception elicited by *p*-phenylquinone. Other signs included forepaw treading/tremors, scream on touch, wet-dog shakes, spontaneous jumping, ptosis, rhinorrhea and hypothermia.

Table 3
Altered sensitivity to opioids in: (A) P42 juvenile rats administered fentanyl, (B) P55 juvenile rats administered morphine and (C) P90 adult rats administered morphine

Treatment	ED_{50} (C.L.) μg kg^{-1}	Potency Ratio (C.L.)
(A) Fentanyl's potency in P42 juvenile rats		
Naive	28.1 (22.2 to 33.9)	–
Saline pump		vs. Naive
(1 μl h^{-1})	32.2 (25.4 to 39.1)	1.11 (0.83 to 1.54)
Fentanyl pump		vs. Saline-P
(50 μg kg^{-1} h^{-1})	32.6 (25.9 to 39.2)	1.10 (0.67 to 1.63)
(B) Morphine's potency in P55 juvenile rats		
Naive	3.9 (3.1 to 4.6)	–
Saline pump		vs. Naive
(1 μl h^{-1})	4.5 (3.8 to 5.2)	1.16 (0.86 to 1.62)
Fentanyl pump		vs. Saline-P
(50 μg kg^{-1} h^{-1})	10.1 (8.0 to 12.2)[a]	2.58 (1.86 to 3.42)[a]
(C) Morphine's potency in P90 adult rats		
Naive	8.9 (8.0 to 9.8)	–
Saline pump		vs. Naive
(1 μl h^{-1})	9.3 (8.2 to 10.5)	1.08 (0.96 to 1.24)
Fentanyl pump		vs. Saline-P
(50 μg kg^{-1} h^{-1})	22.3 (19.9 to 24.7)[a]	2.55 (2.25 to 2.87)[a]

[a]Significantly different from saline pump animals.
P14 rats remained naive or were implanted with osmotic minipumps as described in Table 1. The pumps were removed 5 days later, and the animals matured into P42 juveniles before being challenged with fentanyl. On P55, the juveniles were challenged with morphine. On P90, the animals as adults were challenged with morphine. Antinociception was measured 10 and 30 min after the injection of fentanyl and morphine, respectively.

3.3. Altered antinociceptive sensitivity in juvenile and adult rats

As mentioned earlier, adult rats that were exposed in utero to chronic opiate administration exhibited altered antinociceptive effects to opiates. We hypothesized that juvenile or adult rats exposed to fentanyl as infants would exhibit a diminished antinociceptive response to fentanyl. P14 rats for these studies remained opiate-naive or were infused with fentanyl (50 μg kg^{-1} h^{-1}). On P17, these animals were not challenged with fentanyl or naloxone. As detailed in Section 2, the pumps were removed on P19, and on P21 the rats were housed in pairs until they matured into juveniles. On P42, fentanyl dose–response curves were generated. As seen in Fig. 2 and Table 3A, the potency of fentanyl did not differ significantly among each treatment group. Thus, the hypothesis was not supported that juveniles exposed to fentanyl as infants would be less sensitive to fentanyl. Since it was unlikely that fentanyl's effects would be diminished in these animals as adults, we decided to test morphine because of its lower receptor intrinsic activity. Juvenile animals remained drug-free for

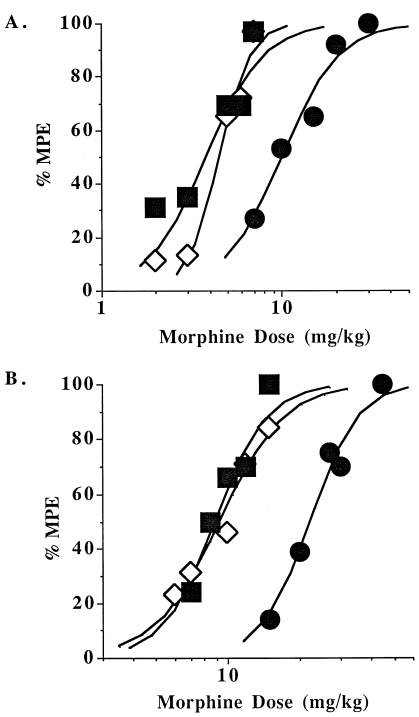

Fig. 3. (A) Rats tolerant to fentanyl as infants are less sensitive than opioid-naive animals to morphine antinociception as juvenile P55 rats. P42 rats from Fig. 2 were allowed to mature to age P55 before challenging them with morphine s.c. for generation of dose–response curves. As infants, these animals remained naive (■) or were infused for 72 h with saline (◇, 1 μl h^{-1}) or fentanyl (●, 50 μg kg^{-1} h^{-1}) from osmotic minipumps. Antinociception was measured 30 min later. (B) Rats tolerant to fentanyl as infants are less sensitive than opioid-naive animals to morphine antinociception as adult P90 rats. P55 rats from the preceding experiment were allowed to mature into adult P90 rats before challenging them with morphine s.c. for generation of dose–response curves. Each dose–response curve represents 20 to 25 rats.

15 days before morphine dose–response curves were generated. On P55, morphine's potency was significantly less in rats that were tolerant to fentanyl as infants (Fig. 3A, Table 3B). It was also important to establish whether morphine's potency would remain diminished into adulthood. Juvenile animals remained drug-free for 35 days as they matured into adults. On P90, the potency of morphine was again significantly lower in rats that were tolerant to fentanyl as infants (Fig. 3B, Table 3C).

4. Discussion

4.1. Fentanyl tolerance and dependence in infant rats

Infant P17 rats were rendered tolerant to fentanyl in a manner similar to an earlier study on P9 rats (Thornton and Smith, 1995). The degree of tolerance was similar, yet the P17 rats became tolerant to a fentanyl dose 50% less than that infused in P9 rats (i.e., 50 vs. 100 $\mu g\ kg^{-1}\ h^{-1}$, respectively). It is noteworthy that fentanyl is more potent in the tail-flick test in P17 rats than P9 rats (Thornton et al., 1998). Thus, P17 rats may be more susceptible to tolerance from smaller infusion doses. The tolerance exhibited in both ages of rats reflects clinical reports of fentanyl tolerance in both preterm and full-term human infants (Maguire and Maloney, 1988; Norton, 1988; Arnold et al., 1990, 1991; French and Nocera, 1994; Katz et al., 1994; Franck and Vilardi, 1995).

This is not the first report of tolerance in neonatal and infant rats. Morphine tolerance is manifested in neonatal rats, but reports disagree about the age at which this can occur. Some reported tolerance in rats at P7 (Van Praag and Frenk, 1991; Barr and Wang, 1992), whereas others did not observe tolerance until the rats were 15-days-old (Fanselow and Cramer, 1988; Windh et al., 1995). In each study, morphine was administered repeatedly by bolus injection using a variety of dosing and injection schedules. The difference in schedules may allow researchers to examine both short- and long-term tolerance (Barr and Wang, 1992). Thus, by adjusting dosage and injection schedules, tolerance can develop within 24 h (Barr et al., 1986), or be delayed by several days. Osmotic minipump infusion has allowed us to examine the consequences of short-term tolerance, which closely mimics reports of human neonatal and infant tolerance occurring in 3 to 5 days of continuous intravenous fentanyl infusion.

Neonatal opiate dependence has also been reported by others. Naloxone precipitated morphine withdrawal has been observed as early as P7, with the repertoire of signs appropriate to the age of the animals (Barr and Wang, 1992; Jones and Barr, 1995; Windh et al., 1995). P17 rats infused with fentanyl also became physically dependent. Fentanyl dependence was also demonstrated in P9 rats, although the signs differed somewhat from P17 rats due to developmental maturity (Thornton and Smith, 1995). Like P17 rats, P9 rats exhibited increases in spontaneous activity, diarrhea, wall climbing, abdominal stretching, tremors, and scream on touch. Yet wet-dog shakes, ptosis, and rhinorrhea were additional signs manifested in P17 rats. In a study of morphine dependence, P17 rats exhibited identical signs to fentanyl dependent P17 rats (Thornton et al., 1997). Thus, the neuronal mechanisms leading to physical dependence are probably shared by fentanyl and morphine.

It could also be argued that the stress of shipping the rat pups contributed to tolerance in the fentanyl infused group. Shipping has been found to delay litter production in female mice, affect cortical thickness and asymmetry in rats exposed prenatally to shipping stress, and affect clinicopathologic indicators in adult rats (Bean-Knudsen and Wagner, 1987; Stewart and Kolb, 1988). Little is known about the influence of shipping on neonatal and infant rats. However, during the first 2 weeks of life, neonatal and infant rats are hypo-responsive to stress (Viau et al., 1996; Halasz et al., 1997). Stress-induced increases in plasma corticosterone and ACTH are blunted, and the expression of corticotropin-releasing factor is decreased. Handling stress that normally elevates β-endorphin and the adrenocortical response in adult rats is absent in animals during the first 2 weeks of life (Iny et al., 1987). Thus, the impact of shipping stress on infant rat development may be minimal. In an earlier study, we characterized fentanyl tolerance in P9 neonatal rats that were born in a breeding colony in our facilities (Thornton and Smith, 1995). The degree of tolerance exhibited in these rats was similar to that in this study.

4.2. Altered antinociceptive sensitivity in juvenile and adult rats

The hypothesis that juvenile rats tolerant to fentanyl as infants would exhibit a reduced antinociceptive response to fentanyl was not demonstrated. However, morphine was significantly less potent in these animals as juveniles and adults. To our knowledge, this is the first report of postnatal fentanyl tolerance resulting in a desensitization to opiates in juvenile and adult rats. Chronic in utero opiate exposure has been clearly shown to alter opiate antinociceptive potency in the offspring. In utero morphine exposure either reduced (O'Callaghan and Holtzman, 1976) or enhanced (Kirby et al., 1982) the antinociceptive potency of morphine. The difference in outcome may depend on dosage, schedule and duration of injections. In another study, in utero methadone exposure increased the potency of morphine in P4 and P21 offspring (Enters et al., 1991). Opiate exposure during the pre- and postnatal period could affect central nervous system (CNS) development differently, with the final outcome represented by an altered antinociceptive response. Comparisons of pre- and postnatal opiate exposure may reveal major neuroanatomical and neurophysiological differences. Thus, the overall conclusion is that both pre- and postnatal opiate exposure alter the antinociceptive response to opiates later in life.

However, it could be argued that challenging rats with fentanyl on P42 affected the subsequent potency of morphine in P55 and P90 rats. However, each treatment group received fentanyl on P42, and yet morphine's potency was reduced in juveniles and adults that were tolerant to fentanyl as infants. The inability to find potency differences among the groups with fentanyl at P42 could reflect its high receptor intrinsic activity. Fentanyl is 100 times more potent than morphine in rats (Thornton et al., 1998). Thus, any group differences may have been obviated by fentanyl's high intrinsic potency and efficacy. Alternatively, the lower intrinsic activity of morphine requires a larger fractional receptor occupancy to elicit antinociception. This was illustrated by Holtzman et al. who utilized the irreversible μ-opioid receptor antagonist β-funaltrexamine to abolish morphine's antinociceptive effects without significantly affecting the potency or efficacy of fentanyl (Adams et al., 1990). Obviously, further studies should examine whether μ-opioid receptor affinity and density are affected, as well as μ-opioid receptor G-protein coupling.

In summary, our results indicate that infant fentanyl exposure reduces the subsequent sensitivity of these animals to opiates later in life. This model may enable researchers to examine the long-term consequences of infant opiate exposure on other behaviors, as well as neurophysiological changes in the CNS.

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