Fentanyl Self-Administration in Juvenile Rats That Were Tolerant and Dependent to Fentanyl as Infants

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THORNTON, S. R., A. B. LOHMANN, R. A. NICHOLSON AND F. L. SMITH. *Fentanyl self-administration in juvenile rats that were tolerant and dependent to fentanyl as infants.* PHARMACOL BIOCHEM BEHAV **65**(3) 563–570, 2000.—Human neonates and infants can become tolerant and dependent during continuous fentanyl or morphine administration. The long-term consequences in these individuals as juveniles and adults are unknown. This study compared fentanyl self-administration behavior in juvenile rats that were opioid naive or were exposed chronically to fentanyl as infants. Postnatal day 14 infant rats remained naive or were implanted with saline- or fentanyl-filled Alzet minipumps. After 72 h, fentanyl's antinociceptive potency was 3.0-fold lower in the fentanyl-infused rats. Naloxone precipitated withdrawal occurred only in the fentanyl-infused animals. Other similarly treated infant rats were allowed to mature into P42 juvenile rats before enrolling them in an oral fentanyl self-administration study. Rats from each group consumed significantly more fentanyl than quinine. However, those rats, tolerant and dependent to fentanyl as infants, did not self-administer more fentanyl than their opiate-naive littermates. The issue of whether fentanyl was consumed for its reinforcing properties was demonstrated when noncontingent administration of opiate antagonists significantly reduced fentanyl intake in another group of juvenile rats. These data indicate that fentanyl is consumed for its reinforcing properties, but that infant fentanyl tolerance and dependence did not predispose them to self-administer more fentanyl than opiate-naive animals.   © 2000 Elsevier Science Inc.

Fentanyl   Opiate   Rat   Self-administration   Neonatal   Analgesia   Tolerance   Physical dependence

CONSIDERABLE evidence indicates that human neonates and infants can develop iatrogenic tolerance and dependence to continuously infused intravenous fentanyl or morphine (1,10,11,20,27,36). To our knowledge, nothing is known about the long-term effects on these patients. More is known about the consequences of in utero opiate exposure. In utero methadone exposure results in low birth-weight infants exhibiting increases in oral sucking drive, and a more rapid motor development (7,33,37). Yet children exposed to methadone in utero achieve normal motor and cognitive developmental milestones at 6 months, and 1 to 4 years of age (19,45). However, these children are at greater risk for developing attention deficit disorder, with accompanying impairment of fine motor coordination (49). Other long-term consequences may be revealed as these infants become juveniles and adults. In like manner, chronic postnatal fentanyl or morphine infusion in human neonates may also affect the ongoing maturation of the CNS.

In rats, the long-term consequences of in utero morphine, methadone, and heroin exposure have been examined. In utero opiate exposure alters opiate receptor density (14,21,48,50), and neural development (13,43,57,58). Behavioral consequences in juvenile and adult rats include an altered sensitivity to opiate antinociception (9,22,36,59), as well as an increased propensity to self-administer IV morphine or heroin (12,17,38).

The hypothesis proposed in this study was that infant rats chronically exposed to fentanyl would self-administer significantly more fentanyl as juveniles than their opiate naive littermates. Fentanyl possesses reinforcing properties sufficient to maintain self-administration. Fentanyl is self-administered IV (28,56), and readily substitutes for IV heroin (24) and dihydroetorphine (28). Furthermore, oral fentanyl self-administration paradigms are sensitive enough to reveal differences in intake between nonarthritic and arthritic rats (4), nonstressed and stressed rats (39–41), and rats without and with neuropathic pain (25). For this study, oral fentanyl self-administration behavior was compared between juvenile rats that were opiate naïve as infants, or were chronically exposed to fentanyl from surgically implanted Alzet osmotic minipumps.

METHOD

Source of Infant Rats

Nulliparous female Sprague–Dawley dams with appropriately aged litters consisting of 10 pups (five females and five males) were purchased from Zivic-Miller (Zedianople, PA). Litter orders were timed to allow a 1-week waiting period in the animal care facilities before enrolling them in the experiments at the age of P14. The animals were housed at the Medical College of Virginia in rooms maintained at 22 ± 1°C with a 12 L:12 D cycle (lights on 0700–1900 h), and food and water were allowed ad lib before the self-administration experiments began. During the self-administration study, fluid requirements were obtained from the quinine and/or fentanyl bottles. Experiments were conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) at the Medical College of Virginia.

Surgical Implantation of Alzet Osmotic Minipumps

Alzet osmotic minipumps were surgically implanted as previously reported (51). Briefly, P14 infant rats were briefly anesthetized with methoxyflurane (Metofane®, Pitman-Moore, Mundelein, IL). Following induction of anesthesia (as noted by the absence of the righting reflex and foot pinch response), the pups were placed on a 37°C heating pad. Fur was depilated with animal shears 1.5 cm from the base of the tail and the area was swabbed with 70% ethanol. Sterile scissors were used to make a 1-cm incision approximately 1.5 cm from the base of the tail. Following the incision, a sterile preloaded 1003D Alzet osmotic minipump (Alza Corp., Palo Alto, CA) was inserted subcutaneously under the skin, and the incision closed with Vetbond Tissue Adhesive (3M Animal Care Products, St. Paul, MN). The 90 μl pump reservoir is guaranteed by Alza to deliver at a rate of 1 μl/h for 72 h. The pump was inserted so that the delivery port was 180° from the incision to prevent drug leakage from the incision. The area was swabbed with 10% providone iodine (General Medical Corp., Prichard, WV), and the animal was allowed to recover. The animals were injected IP with 60,000 U of potassium penicillin G (various suppliers) to prevent infection and 0.5 ml of isotonic saline SC to prevent hypovolemia according to IACUC guidelines at the Medical College of Virginia. 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. The pups were returned to the dam and 72 h later on P17 were challenged with fentanyl SC to assess antinociception, or naloxone to assess

for physical dependence. Other animals were assigned to the self-administration protocol without being challenged with drug on P17.

Tail-Flick Test

Antinociception was measured in naïve P17 rats, and P17 rats chronically infused for 72 h with saline or fentanyl (50 μg/kg/h). The tail-flick test was developed by D'Amour and Smith (6) and modified by Dewey et al. (8). Before injections, the baseline (control) latency for each rat was measured. The intensity of the heat stimulus was adjusted to yield baseline latencies of 3 to 4 s, with a 10-s cutoff to prevent tissue damage (9,31,51). The peak time of antinociception is 10 min after SC administration (52). At the peak time of 10 min, fentanyl antinociception was quantified according to the method of Harris and Pierson (15) as the percentage of maximum possible effect (% MPE) which was calculated as: $\%MPE = [(\text{test} - \text{control})/(10 - \text{control})] \times 100$. ED_{50} values were calculated using least-squares linear regression analysis of %MPE values followed by calculation of 95% confidence limits (CL) according to the method of Bliss (2). Dose–response curves were considered significantly different if the 95% CLs did not overlap. Tests for parallelism were conducted before calculation of potency ratio values and 95% confidence limits by the method of Colquhoun (5). A potency ratio value greater than 1, with lower 95% confidence limits greater than 1, was considered a significant difference in potency.

Withdrawal Testing

Naloxone (5 mg/kg, SC) was administered to naive P17 rats, and P17 rats chronically infused for 72 h with saline or fentanyl (50 μg/kg/h). After 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 signs were quantified as the number exhibiting the sign to the total number of animals observed. These signs included: micturation/defecation, face washing, wall climbing, abnormal posture, forepaw treading/tremors, scream on touch, wet-dog shakes, spontaneous jumping, mastication, ptosis, rhinorrhea, and changes in body temperature.

Surgical Removal of Alzet Osmotic Minipumps

For the self-administration studies, osmotic minipumps were removed on P19. Alza guarantees the 1003D pump to deliver drug at a rate of 1 μl/h for 72 h. The pump remained in the animals 2 additional days before removal from the animals. Portions of the remaining 18 μl are thought to be delivered over the next 12 h, although delivery rates have not been verified by Alza. On P19, the rats were briefly anesthetized with methoxyflurane. 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 and swabbed with iodine. The animals received 60,000 U of potassium penicillin G IP to prevent infection. The animals were ear-tagged for identification and returned to the dam. Forty-eight hours later at P21 they were weaned and housed in groups of two per cage

until P42, when they were then entered into the oral fentanyl self-administration paradigm.

Oral Fentanyl Self-Administration

The oral self-administration protocol was a modification of a two-bottle free choice paradigm described by Sudakov et al. (47). On session day 0, P42 animals were water deprived for 24 h, and then offered quinine solution from two bottles for 90 min twice daily for session days 1 to 9 (P43 to P51). During these "forced" trials the quinine concentrations were increased over the 9 days as follows: 7 μg/ml (days 1 to 3), 13 μg/ml (days 4 to 6), and 25 μg/ml (days 7 to 9). This conditioning acclimated the animals to the alkaloid taste of quinine matched to that of fentanyl. On session day 10 (P52) the rats were given the choice of drinking quinine (0.0025% = 25 μg/ml) or fentanyl (25 μg/ml) from two bottles twice daily for the next 9 days (P52 to P60). The fentanyl bottles were placed on either the left or right side of the cage to prevent a position preference. During the "forced" and "choice" trials the animals were allowed to consume solution twice daily for 90 min (08008 and 2000 h). The bottles and animals were weighed daily to calculate the fentanyl dose (μg/kg), and the volumes of quinine and fentanyl consumed each day. The data were analyzed using repeated-measures ANOVA, followed by the Tukey's test for post hoc comparisons among the treatment groups. On session day 19 (P61), the rats from each postnatal treatment group were administered naloxone (5 mg/kg, SC) and assessed for signs of physical dependence to the consumed fentanyl.

Influence of Opiate Antagonists on Self-Administration

Another group of juvenile P42 animals were were water deprived for 24 h, and then offered quinine solution from two bottles for 90 min twice daily for "forced" session days 1 to 9 (P43 to P51). On session day 10 (P52), the rats were given a choice of drinking quinine (0.0025%; 25 μg/ml) or fentanyl (25 μg/ml) from two bottles twice daily for session days 10 to 22 (P52 to P64). On session days 15 and 20, the rats received naloxone and naltrexone, respectively, 15 min before giving them access to the fentanyl, and quinine bottles in both the morning and evening sessions.

Drugs and Solutions

Crystalline fentanyl hydrochloride (National Institute on Drug Abuse, Bethesda, MD) was dissolved in sterile pyrogen-free isotonic saline (Baxter Healthcare Corp., Deerfield, IL). Osmotic minipumps were filled with either fentanyl or isotonic saline. For self-administration studies, fentanyl hydrochloride (NIDA) and quinine hydrochloride (Sigma Chemical Co., St. Louis, MO) were dissolved in distilled water. Naloxone hydrochloride was dissolved in saline (Research Biochemicals International, Natick, MA).

RESULTS

Fentanyl Tolerance in P17 Rats

For these experiments P14 rats remained naive or were surgically implanted with Alzet 1003D osmotic minipumps as detailed in the Method section. At 72 h, baseline tail-flick latencies were not different among the groups, indicating that the fentanyl infused from the pump did not elicit antinociception (data not shown). However, the potency of acutely ad-
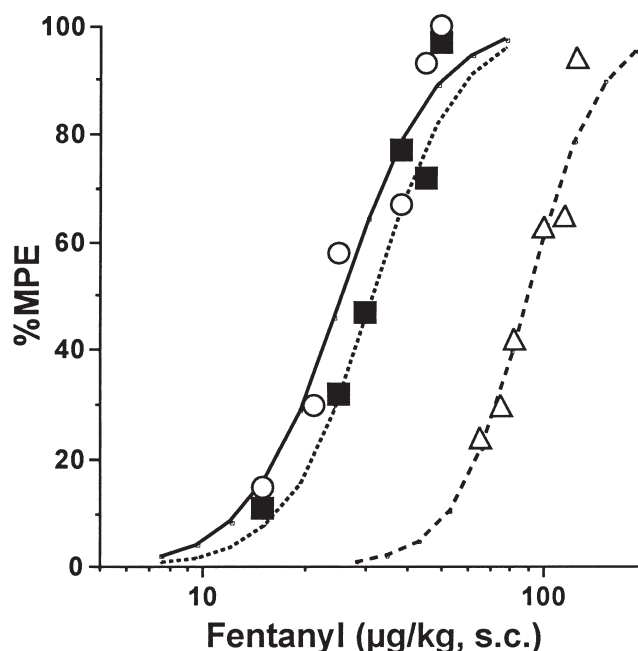

FIG. 1. Tolerance to the antinociceptive effects of fentanyl in postnatal day 17 (P17) rats. P14 rats remained naive or were implanted with 1003D Alzet osmotic minipumps that infused saline (1 μl/h) or fentanyl (50 μg/kg/h) for 72 h. At P17, baseline tail-flick latencies were obtained before administering fentanyl SC to naive ($\bigcirc$), saline- ($\blacksquare$, 1 μl/h) or fentanyl-infused ($\triangle$, 50 μg/kg/h) P17 rats. Ten minutes later, test latencies were obtained for calculation of %MPE. Each dose–response curve represents 30 rats.

ministered fentanyl was significantly reduced in fentanyl-infused rats compared to saline-infused rats (Fig. 1). Not only were the ED$_{50}$ values increased (Table 1), but potency ratio calculations revealed a 3.0-fold decrease in the potency of fentanyl. It is noteworthy that the potency of fentanyl in saline-pump implanted rats was the same as naive animals, which indicated the absence of a pump effect on antinociception. These animals were not used in the fentanyl self-administration study.

TABLE 1
TOLERANCE TO FENTANYL IN P17 RATS AFTER A 72-h INFUSION OF FENTANYL (50 μg/kg/h) FROM OSMOTIC MINIPUMPS

Treatment	ED$_{50}$ μg/kg (95% C.L.)	Potency Ratio (95% C.L.)
Naive	25.7 (21.6 to 29.7)	—
Saline pump (1 μl/h)	31.6 (27.2 to 40.0)	vs. naive 0.88 (0.74 to 1.04)
Fentanyl pump (50 μg/kg/h)	90.1 (80.6 to 99.5)*	vs. saline-P 3.01 (2.60 to 3.50)†

P14 rats remained naive or were implanted with Alzet osmotic minipumps that infused saline or fentanyl (50 μg/kg/h) for 72 h. At P17 the rats were tested for antinociception in the tail-flick test 10 min after SC administration of fentanyl. n = 30 rats per dose–response curve.

*Significantly different from saline pump group, Bliss (2)
†Significantly different from saline-pump group, Colquhoun (5).

Physical Dependence to Fentanyl in P17 Rats

The hypothesis was tested that continuous fentanyl administration renders P17 rats physically dependent to fentanyl. Fentanyl-infused P17 rats administered naloxone (5 mg/kg, SC) displayed a precipitated withdrawal syndrome similar to that observed in adult rats physically dependent to opiates (Table 2). Fentanyl-infused rats displayed the highest level of spontaneous activity, which often resulted in wall climbing behavior and spontaneous jumping. Although all the animals micturated and defecated in the open field, only the fentanyl-infused rats exhibited diarrhea. Half of the fentanyl-infused rats exhibited a decrease in face washing. Half exhibited spontaneous auditory vocalization, and expressed a high-pitch scream with tactile stimulation. Other signs in 75 to 90% of the fentanyl-infused rats were abnormal posture, and abdominal stretching similar to the visceral nociception elicited by *p*-phenylquinone. All fentanyl-infused rats exhibited wet-dog shakes, forepaw treading/tremors, ptosis, rhinorrhea, and hypothermia. These animals were not used in the fentanyl self-administration study.

Oral Fentanyl Self-Administration in Juvenile Rats

Opiate-naïve and fentanyl-infused P17 rats not challenged with fentanyl or naloxone were reared into juveniles (P42) before being tested in the oral fentanyl self-administration paradigm. The hypothesis was tested that juvenile rats chronically exposed to fentanyl as infants would be predisposed to self-administer more fentanyl than their opiate-naive littermates. As seen in Fig. 2A, quinine was offered from two bottles for 90 min twice daily during the "forced" session days 1 to 9 (ages P43 to P51). On session day 10 (P52), the rats were

given the choice of drinking quinine (0.0025% = 25 μg/ml) or fentanyl (25 μg/ml) from two bottles twice daily during "choice" session days 10 to 18 (P52 to P60). Repeated-measures ANOVA of quinine intake over session days 1 to 18 indicated no significant difference between treatment groups on intake, $F(2, 39) = 0.50$, $p = 0.613$. However, there was a significant treatment × day interaction, $F(2, 34) = 1.90$, $p = 0.018$. Post hoc analysis with the Tukey's test ($W_r = 8.2$ ml) indicates that quinine intake declined significantly in all treatment groups during the "choice" session days 10 to 18. On session day 10 the rats consumed roughly half their daily intake from both the quinine and fentanyl bottles. However, on successive days the animals increased the percentage of fentanyl consumed, with concomitant reductions in quinine intake. In fact, by day 12 approximately 75% of the daily fluid intake was obtained from the fentanyl bottle. These results indicate that the rats from each treatment group preferred consuming the fentanyl solution.
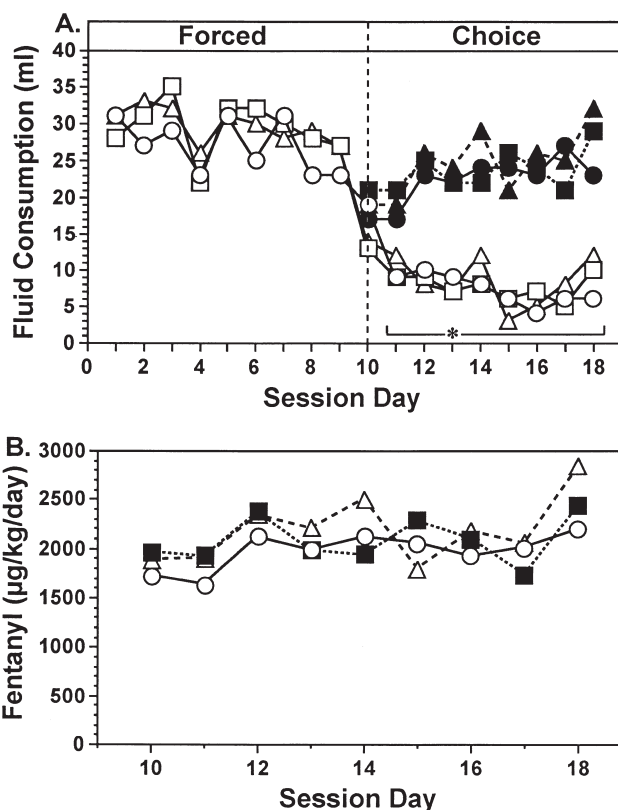

FIG. 2. (A) Daily consumption of quinine and fentanyl in juvenile rats during the "forced" and "choice" sessions. P42 animals water deprived for 24 h were offered quinine solution from two bottles on "forced" session days 1 to 9. For "choice" session days 10 to 18, the rats were allowed to drink from quinine or fentanyl (25 μg/ml) bottles. Animals that remained naive at P17 consumed quinine (○) and fentanyl (●). Animals that were saline infused at P17 consumed quinine (■) and fentanyl (■), while animals that were fentanyl tolerant at P17 consumed quinine (△) and fentanyl (▲). Group sizes were: naive ($n = 16$), saline-infused ($n = 12$) or fentanyl-infused ($n = 17$). (B) Influence of neonatal fentanyl exposure on daily fentanyl dose consumed in juvenile rats. The daily dose of fentanyl consumed from session days 10 to 18 (P52 to P60) is represented in juvenile rats that were naive (○), saline infused (■) or fentanyl infused (△) as infants. The group sizes are represented in (2A).

TABLE 2

NALOXONE PRECIPITATED WITHDRAWAL IN P17 RATS AFTER A 72-h INFUSION OF FENTANYL (50 μg/kg/h) FROM OSMOTIC MINIPUMPS

Withdrawal Signs	Naive	Saline-P	Fentanyl-P
Spontaneous activity	156 lines/ animal	203 lines/ animal	334 lines/ animal*
Micturation/defecation	5/9 6/9	8/9 8/9	9/9 9/9‡
Face washing	9/9	9/9	4/9
Wall climbing	0/9	0/9	5/9†
Abnormal posture	0/9	0/9	7/9†
Forepaw treading/ tremors	0/9	0/9	9/9†
Scream on touch	0/9	0/9	5/9†
Wet-dog shakes	0/9	0/9	6/9†
Spontaneous jumping	0/9	0/9	6/9†
Mastication	7/9	6/9	8/9
Ptosis	0/9	0/9	9/9†
Rhinorrhea	0/9	0/9	9/9†
Change in body temperature (°C)	−0.06	−0.42	−1.8*

P14 rats remained naive or were implanted with Alzet osmotic minipumps that infused saline or fentanyl (50μg/kg/h) for 72 h. At P17, rats administered naloxone (5 mg/kg, SC) were observed for 15 min for signs of physical dependence.

*Significantly different from saline-pump animals, ANOVA, Tukey's test.

†Significantly different from saline-pump animals, chi square.

‡Diarrhea.

Regarding the dose of fentanyl self-administered during session days 10 to 18, the magnitude dose consumed (μg/kg) did not differ significantly between the treatment groups over time, $F(2, 16) = 1.62$, $p = 0.06$ (Fig. 2B). However, the average daily fentanyl dose increased 37% from session days 10 to 18. Thus, juvenile rats that were tolerant to fentanyl as infants were not predisposed to self-administer more fentanyl than their opiate-naive littermates.

Measurements of total daily fluid intake and weight gain were collected and analyzed to demonstrate that fentanyl consumption did not affect these parameters. As seen in Fig. 3A, the switch from the "forced" to the "choice" days did not affect the daily volume consumed in any group. Furthermore, the introduction of fentanyl on session day 10 did not subsequently affect weight gain (Fig. 3B). Over 18 days the animals gained approximately 100 g, at an average increase of 5.6 g/day.
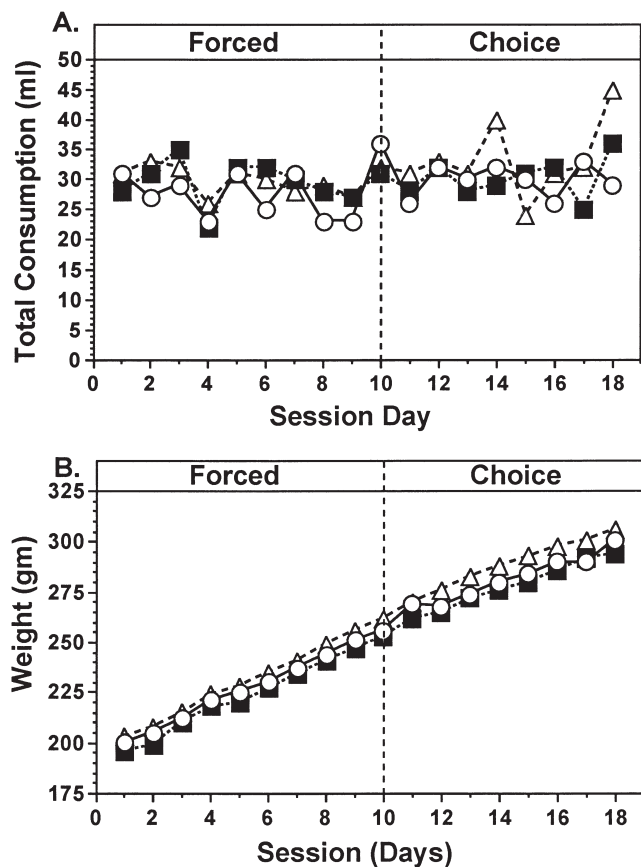

FIG. 3. (A) Influence of fentanyl consumption on daily volume intake during the "choice" sessions. On "forced" session days 1 to 9, juvenile rats consumed quinine, as described in Fig. 2A. On "choice" session days 10 to 18 the animals consumed quinine or fentanyl from two bottles. Daily volume consumed is represented in juvenile rats that were naive (○), saline infused (■) or fentanyl infused (△) as infants. Group sizes are represented in Fig. 2A. (B) Influence of fentanyl consumption on weight gain during the "choice" sessions. Session days 1 to 9 represent weight gain in juvenile rats consuming quinine solutions. Session days 10 to 18 represent weight gain in animals given the "choice" of consuming quinine or fentanyl from two bottles. Weight gain is represented in juvenile rats that were naive (○), saline infused (■) or fentanyl infused (△) as infants.

TABLE 3

NALOXONE PRECIPITATED WITHDRAWAL IN P61 RATS AFTER 9 DAYS OF FENTANYL SELF-ADMINISTRATION

Withdrawal Signs	Naive	Saline-P	Fentanyl-P
Micturation/defecation	7/7 1/7	5/5 0/5	8/8 0/8
Face washing	6/7	5/5	8/8
Excessive grooming	5/7	5/5	8/8
Abnormal posture	7/7	4/5	7/8
Forepaw tremors	6/7	5/5	3/8
Teeth chattering	6/7	2/5	2/8
Wet-dog shakes	4/7	5/5	5/8
Mastication	6/7	5/5	6/8
Spontaneous jumping	0/7	0/5	0/8
Ptosis	0/7	0/5	0/8
Rhinorrhea	0/7	0/5	0/8

P61 rats injected with naloxone (5 mg/kg, SC) were observed 15 min for signs of physical dependence after 9 days of fentanyl self-administration, as described in the Method section.

It could be argued that the daily fentanyl dose was too low to elicit pharmacological effects. Therefore, on session day 19 P61 animals from each treatment received naloxone and were assessed for signs of physical dependence. As seen in Table 3, the animals from each treatment group consuming fentanyl showed signs of mild dependence. Some signs not observed were diarrhea, spontaneous jumping, ptosis, and rhinorrhea. Thus, the daily self-administered fentanyl dose was sufficient to elicit pharmacological effects.

Another argument could be that the rats were not consuming fentanyl for its reinforcing properties, but instead were consuming a less aversive alkaloid solution than quinine. Juvenile rats in another study, with no prior infant opiate exposure, were allowed to consume quinine or fentanyl. On two separate days, opiate receptor antagonists were injected noncontingently 15 min before allowing them access to quinine

TABLE 4

INFLUENCE OF OPIOID RECEPTOR ANTAGONISTS ON FENTANYL SELF-ADMINISTRATION BEHAVIOR IN JUVENILE RATS

Treatment Days	Fentanyl (μg/kg/day)	Fentanyl Volume (ml/day)	Quinine Volume (ml/day)
Day 14	2308 ± 105	22 ± 2	8 ± 2
Day 15 naloxone (1.0 mg/kg, SC)	1376 ± 83*	13 ± 1*	11 ± 6
Day 16	2760 ± 158	27 ± 2	5 ± 1
Day 19	2300 ± 73	24 ± 2	5 ± 1
Day 20 naltrexone (0.1 mg/kg, SC)	1144 ± 111*	12 ± 1*	4 ± 1
Day 21	2526 ± 123	26 ± 2	4 ± 1

P42 animals were offered quinine solution from two bottles during the "forced" session days 1 to 9 (P43 to P51). On session day 10 (P52), the rats were given a choice of drinking quinine (0.0025%; 25 μg/ml) or fentanyl (25 μg/ml) for session days 10 to 22 (P52 to P64). On session days 15 and 20, the rats received naloxone and naltrexone, respectively, 15 min before giving them access to the fentanyl and quinine bottles in both the morning and evening sessions. $n = 10$ rats.

$p < 0.05$, compared to respective intake on the previous day, ANOVA, and Tukey's test.

and fentanyl. Naloxone (1 mg/kg, SC) was injected on session day 15, and naltrexone (0.1 mg/kg, SC) on session day 20 (Table 4). ANOVA revealed a significant effect of opiate antagonist on daily intake, $F(11, 132) = 7.43$, $p = 0.001$. On session day 15, naloxone significantly reduced both the fentanyl dose and volume consumed by 40% compared to the previous day, while increasing the quinine volume consumed by 38%. On session day 20, naltrexone decreased both the fentanyl dose and volume consumed by 50%, with no real effect on quinine consumption.

DISCUSSION

Our results indicate that P14 rats implanted with Alzet osmotic minipumps infusing fentanyl (50 μg/kg/h) were rendered tolerant and dependent 72 h later. P17 rats were tolerant to the acute antinociceptive effects of fentanyl, and animals injected with naloxone showed signs of physical dependence. The extent of tolerance is similar to an earlier report on P9 rats (51). However, the signs of dependence were more complex in P17 than P9 rats, reflecting the developmental maturation of behaviors throughout the preweanling period. In fact, P17 rats exhibited a profile of dependence similar to that observed in juvenile rats (Table 2 vs. Table 3). Thus, preweanling rats at different postnatal ages are susceptible to the chronic effects of fentanyl. This is similar to reports of opiate tolerance and dependence occurring in preterm neonates and older infants (1,10,11,20,27,42).

As mentioned earlier, P17 rats not tested for tolerance and dependence were allowed to self-administer fentanyl as juvenile rats. We utilized the classic two-bottle technique developed by Meyers (32), and modified by Sudakov (47). The final percent quinine concentration was based on a study of the equa-palatability of quinine and opiate solutions (55). Our results indicate that the juvenile rats from each postnatal treatment group readily consumed the fentanyl solution during the "choice" sessions. Fentanyl has been shown to possess positive reinforcing properties. Rats under different operant conditions of schedule and dose reliably self-administer fentanyl by intravenous injection (56). Adult rats also reliably consume fentanyl offered from bottles, or consume fentanyl during lever pressing under fixed-ratio schedules (23,25,39–41). Yet, although all the juvenile rats consumed fentanyl, the rats with infant fentanyl exposure did not self-administer significantly more fentanyl than their littermates. In contrast, in utero opiate exposure increases the amount of opiate self-administered in adulthood. Heroin IV self-administration was significantly greater in rats that were exposed in utero to morphine (38). Another group found that in utero methadone predisposed the adult rat offspring to self-administer more morphine orally than control offspring (17). Finally, in utero morphine facilitated the rate at which morphine self-administration was learned (12). It could be argued that intravenous self-administration can reveal more subtle differences than oral intake paradigms. Yet the study by Hovious and Peter (17) showing in utero effects of methadone was conducted in

adult animals consuming morphine. Differences in fentanyl doses consumed have been detected between animal strains, in nonstressed and stressed rats, and in animals with chronic pain conditions (4,25,39–41,47).

However, it could be argued that fentanyl was chosen as a less aversive solution rather than being consumed for its reinforcing properties. If fentanyl was not reinforcing, then noncontingent administration of opiate receptor antagonists would not affect fentanyl intake. Yet both naloxone and naltrexone significantly reduced the consumption of fentanyl, followed by a normalization of intake 1 day later (Table 3). Both central and peripheral administration of opiate receptor antagonists has been shown to block opiate reinforcement, and significantly modify self-administration behavior (29,34, 53,54).

Interestingly, most studies of fentanyl consumption reveal that oral doses are significantly higher than intravenous doses. In our study, daily fentanyl intake ranged from 1600 to 2800 μg/kg/day. Oral fentanyl undergoes first-pass conversion by intestinal and hepatic P450 3A into inactive metabolites. P450 3A is present in animals as young as P4 (3). N-Dealkylation of fentanyl into nor-fentanyl is the major route of metabolism of fentanyl and related phenylpiperidines (18,26,30,42). Another pathway involves amide hydrolysis of fentanyl into despropionyl fentanyl, which is subsequently converted by N-dealkylation into despropionyl nor-fentanyl (18). It could be argued that oral administration did not provide enough unmetabolized fentanyl for reinforcement. However, the lipophilicity of fentanyl can lead to significant absorption through the mucosa of the mouth, pharynx, and esophagus to induce pharmacological effects. Stanley et al. (44) demonstrated that surgical premedication with a fentanyl citrate lollipop provided effective sedation, analgesia, respiratory depression, and other common opiate side effects. Peak plasma levels of oral transmucosal fentanyl reflect concentrations required for analgesia (46). In one case of dependence, the individual who ingested liquid fentanyl experienced euphoria, and had unchanged fentanyl urine concentrations of 1 ng/ml (16). In our hands, gavaging rats with a 25-μg/kg dose resulted in significant antinociception (data not shown). Finally, the presence of mild naloxone precipitated withdrawal in each self-administration group at P61 further supports the contention that pharmacologically active doses of fentanyl were consumed (Table 3).

In summary, our results demonstrate that juvenile rats readily self-administer fentanyl. The results with naloxone and naltrexone indicate that consumption was motivated by the rewarding properties of fentanyl. However, juvenile rats with a history of chronic fentanyl exposure in infancy were not predisposed to self-administer more fentanyl than their opiate-naive littermates.

REFERENCES

1. Arnold, J. H.; Truog, R. D.; Orav, E. J.; Scavone, J. M.; Hershenson, M. B.: Tolerance and dependence in neonates sedated with fentanyl during extracorporeal membrane oxygenation. Anesthesiology 73:1136–1140; 1990.
2. Bliss C.I.: Statistics in biology. New York: McGraw-Hill Publishers; 1967:439.
3. Borlakoglu, J. T.; Scott, A.; Henderson, C. J.; Wolf, C. R.: Expression of P450 isoenzymes during rat liver organogenesis. Int. J. Biochem. 25:1659–1668; 1993.
4. Colpaert, F. C.; Meert, T.; De Witte, P.; Schmitt, P.: Further evidence validating adjuvant arthritis as an experimental model of chronic pain in the rat. Life Sci. 31:67–75; 1982.

5. Colquhoun, D.: Lectures in biostatistics: An introduction to statistics with applications in biology and medicine. London: Oxford Clarendon Press; 1971:327–332.
6. D'Amour, F. E.; Smith, D. L.: A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72:74–79; 1941.
7. Davis, M. M.; Shanks, B.: Neurological aspects of perinatal narcotic addiction and methadone treatment. Addict. Disord. 2:213–226; 1975.
8. Dewey, W. L.; Harris, L. S.; Howes, J. F.; Nuite, J. A.: The effect of various neurohormonal modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. J. Pharmacol. Exp. Ther. 175:435–442; 1970.
9. Enters, E. K.; Guo, H.; Pandey, U.; Ko, D.; Robinson, S. E.: The effect of prenatal methadone exposure on development and nociception during the early postnatal period of the rat. Neurotoxicol. Teratol. 13:161–166; 1991.
10. Franck, L.; Vilardi, J.: Assessment and management of opioid withdrawal in ill neonates. Neonatal Network 14:39–48; 1995.
11. French, J. P.; Nocera, M.: Drug withdrawal symptoms in children after continuous infusion of fentanyl. J. Pediatr. Nursing 9:107–109; 1994.
12. Glick, S. D.; Strumpf, A. J.; Zimmerberg, B.: Effect of in utero administration of morphine on the subsequent development of self-administration behavior. Brain Res. 132:194–196; 1977.
13. Hammer, R. P.; Hauser, K. F.: Consequences of early exposure to opioids on cell proliferation and neuronal morphogenesis. In: Miller, M. W., ed. Development of the central nervous system: Effects of alcohol and opiates. New York: Wiley-Liss; 1992.
14. Hammer, R. P.; Seatriz, J. V.; Ricalde, A. R.: Regional dependence of morphine-induced μ-opiate receptor down-regulation in perinatal rat brain. Eur. J. Pharamacol. 209:253–256; 1991.
15. Harris, L. S.; Pierson, A. K.: Some narcotic antagonists in the benzomorphan series. J. Pharmacol. Exp. Ther. 143:141–148; 1964.
16. Hays, L. R.; Stillner, V.; Littrell, R.: Fentanyl dependence associated with oral ingestion. Anesthesiology 77:819–820; 1992.
17. Hovious, J. R.; Peters, M. A.: Opiate self-administration in adult offspring of methadone-treated female rats. Pharmacol. Biochem. Behav. 22:949–953; 1985.
18. Hug, C. C.; Murphey, M. R.: Tissue redistribution of fentanyl and termination of its effects in rats. Anesthesiology 55:369–375; 1981.
19. Kaltenbach, K.; Finnegan, L. P.: Neonatal abstinence syndrome, pharmacotherapy and developmental outcome. Neurobehav. Toxicol. Teratol. 8:353–355; 1986.
20. Katz, R.; Kelly, H. W.; Hsi, A.: Prospective study on the occurrence of withdrawal in critically ill children who receive fentanyl by continuous infusion. Crit. Care Med. 22:763–767; 1994.
21. Kirby, M. L.; Aronstam, R. S.: Levorphanol-sensitive [³H]-naloxone binding in developing brainstem following prenatal morphine exposure. Neurosci. Lett. 35:191–195; 1983.
22. Kirby, M. L.; DeRossett, S. E.; Holtzman, S. G.: Enhanced analgesic response to morphine in adult rats exposed to morphine prenatally. Pharmacol. Biochem. Behav. 17:1161–1164; 1982.
23. Klein, L. C.; Popke, E. J.; Grunberg, N. E.: Sex differences in effects of predictable and unpredictable footshock on fentanyl self-administration in rats. Exp. Clin. Psychopharmacol. 5:99–106; 1997.
24. Koob, G. F.; Vaccarino, F. J.; Amalric, M.; Bloom, F. E.: Neurochemical substrates for opiate reinforcement. NIDA Res. Monogr. 71:146–1464; 1986.
25. Kupers, R.; Gybels, J.: The consumption of fentanyl is increased in rats with nociceptive but not neuropathic pain. Pain 60:137–141; 1995.
26. Lavrijsen, K.; Van Houdt, J.; Van Dyck, D.; Hendrickx, J.; Hurkmans, R.; Bockx, M.; Janssen, C.; Meuldermans, W.; Heykants, J.: Biotransformation of sufentanil in liver microsomes of rats, dogs, and humans. Drug Metab. Dispos. 18:704–710; 1990.
27. Maguire, D. P.; Maloney, P.: A comparison of fentanyl and morphine use in neonates. Neonatal Network 7:27–35; 1988.
28. Martin, T. J.; Kim, S. A.; Harris, L. S.; Smith, J. E.: Potent reinforcing effects of dihydroetorphine in rats. Eur. J. Pharmacol. 324:141–145; 1997.
29. Martin, T. J.; Walker, L. E.; Sizemore, G. M.; Smith, J. E.; Dworkin, S. I.: Within-session determination of dose–response curves for heroin self-administration in rats: Comparison with between-session determination and effects of naltrexone. Drug Alcohol Depend. 41:93–100; 1996.
30. Mautz, D. S.; Labroo, R.; Kharasch, E. D.: Determination of alfentanil and noralfentanil in human plasma by gas chromatography-mass spectrometry. J. Chromatogr. 658:149–153; 1994.
31. McLaughlin, C. R.; Dewey, W. L.: A comparison of the antinociceptive effects of opioid agonists in neonatal and adult rats in phasic and tonic nociceptive tests. Pharmacol. Biochem. Behav. 49:1017–1023; 1994.
32. Myers, A. K.: Alcohol choice in Wistar and G-4 rats as a function of environmental temperature and alcohol concentration. J. Comp. Physiol. Psychol. 55:606–609; 1962.
33. Naeye, R. L.; Blanc, W.; Leblanc, W.; Khatamee, M. A.: Fetal complications of maternal heroin addiction: Abnormal growth, infection and episodes of stress. J. Pediatr. 83:1055–1061; 1973.
34. Negus, S. S.; Henriksen, S. J.; Mattox, A.; Pasternak, G. W.; Portoghese, P. S.; Takemori, A. E.; Weinger, M. B.; Koob, G. F.: Effect of antagonists selective for mu, delta and kappa opioid receptors on the reinforcing effects of heroin in rats. J. Pharmacol. Exp. Ther. 265:1245–1252; 1993.
35. Norton, S. J.: After-effects of morphine and fentanyl analgesia: A retrospective study, Neonatal Network 7:25–28; 1988.
36. O'Callaghan, J. P.; Holtzman, S. G.: Prenatal administration of levorphanol or dextrorphan to the rat: Analgesic effect of morphine in the offspring. J. Pharmacol. Exp. Ther. 200:255–262; 1976.
37. Ramer, C. M.; Lodge A.: Neonatal addiction, part I: Clinical and developmental characteristics of infants of mothers on methadone maintenance. Addict. Disord. 2:227–234; 1975.
38. Ramsey, N. F.; Niesink, R. J.; Van Ree, J. M.: Prenatal exposure to morphine enhances cocaine and heroin self-administration in drug-naïve rats. Drug Alcohol Depend. 33:41–51; 1993.
39. Shaham, Y.: Immobilization stress-induced oral opioid self-administration and withdrawal in rats: Role of conditioning factors and the effect of stress on "relapse" to opioid drugs. Psychopharmacology (Berlin) 111:477–485; 1993.
40. Shaham, Y.; Alvares, K.; Nespor, S. M.; Grunberg, N. E.: Effect of stress on oral morphine and fentanyl self-administration in rats. Pharmacol. Biochem. Behav. 41:615–619; 1992.
41. Shaham, Y.; Klein, L. C.; Alvares, K.; Grunberg, N. E.: Effect of stress on oral fentanyl consumption in rats in operant self-administration paradigm. Pharmacol. Biochem. Behav. 46:315–322; 1993.
42. Silverstein, J. H.; Rieders, M. F.; McMullin, M.; Schulman, S.; Zahl, K.: An analysis of the duration of fentanyl and its metabolites in urine and saliva. Anesth. Analg. 76:618–621; 1993.
43. Smith, A. A.; Hui, F. W.; Crofford, M. J.: Inhibition of growth in young mice treated with d,l-methadone. Eur. J. Pharmacol. 43:307–314; 1977.
44. Stanley, T. H.; Hague, B.; Mock, D. L.; Streisand, J. B.; Bubbers, S.; Dzelzkalns, R. R.; Bailey, P. L.; Place, N. L.; East, K. A.; Ashburn, M. A.: Oral transmucosal fentanyl citrate (lollipop) premedication in human volunteers. Anesth. Analg. 69:21–27; 1989.
45. Strauss, M. E.; Starr, R. H.; Ostrea, E. M.; Chavez, C. J.; Stryker, J. C.: Behavioral concomitants of prenatal addiction to narcotics. J. Pediatr. 89:842–845; 1976.
46. Streisand, J. B.; Varvel, J. R.; Stanski, D. R.; Le-Maire, L.; Ashburn, M. A.; Hague, B. I.; Tarver, S. D.; Stanley, T. H.: Absorption and bioavailability of oral transmucosal fentanyl citrate. Anesthesiology 75:223–229; 1991.
47. Sudakov, S. K.; Goldberg, S. R.; Borisova, E. V.; Surkova, L. A.; Turina, I. V.; Rusakov, D. J.; Elmer, G. I.: Differences in morphine reinforcement property in two inbred rat strains: Associations with cortical receptors, behavioral activity, analgesia and the cataleptic effects of morphine. Psychopharmacology (Berlin) 112:183–188; 1993.
48. Tempel, A.: Visualization of opiate receptor downregulation following morphine treatment in neonatal rat brain. Dev. Brain Res. 64:19–26; 1991.
49. Tempel, A.: Regulation of the opioid system by exogenous drug

administration. In: Miller, M. W., ed. Development of the central nervous system: Effects of alcohol and opiates. New York: Wiley-Liss, Inc.; 1992.

50. Tempel, A.; Haba, J.; Paredes, W.; Barr, G. A.: Morphine-induced down-regulation of μ-opioid receptors in neonatal rat brain. Dev. Brain Res. 41:129–133; 1988.

51. Thornton, S. R.; Smith, F. L.: Characterization of neonatal rat fentanyl tolerance and dependence. J. Pharmacol. Exp. Ther. 281:514–521; 1997.

52. Thornton, S. R.; Compton, D. R.; Smith, F. L.: Ontogeny of mu opioid agonist antinociception in postnatal rats. Dev. Brain Res. 105:269–276; 1998.

53. Vaccarino, F. J.; Pettit, H. O.; Bloom, F. E.; Koob, G. F.: Effects of intracerebroventricular administration of methyl naloxonium chloride on heroin self-administration in the rat. Pharmacol. Biochem. Behav. 23:495–498; 1985.

54. Vaccarino, F. J.; Bloom, F. E.; Koob, G. F.: Blockade of nucleus accumbens opiate receptors attenuates intravenous heroin reward in the rat. Psychopharmacology (Berlin) 86:37–42; 1985.

55. Van Ree, J. M.; Niesink, R. J. M.: Pituitary–adrenal axis and oral morphine consumption in rats. Pharmacol. Biochem. Behav. 9:493–498; 1978.

56. Van Ree, J. M.; Slagen, J. L.; DeWeid, D.: Intravenous self-administration of drugs in rats. J. Pharmacol. Exp. Ther. 204:547–557; 1978.

57. Zagon, I. M.; McLaughlin, P. J.: The effect of chronic morphine adminstration in pregnant female rats and their offspring. Pharamacology 15:302–310; 1977.

58. Zagon, I. M.; McLaughlin, P. J.: Morphine and brain growth retardation in the rat. Pharmacology 15:276–282; 1977.

59. Zagon, I. M.; McLaughlin, P. J.: Protracted analgesia in young and adult rats perinatally exposed to methadone. Life Sci. 29:1137–1142; 1981.