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A Comparison of the Antinociceptive Effects of Opioid Agonists in Neonatal and Adult Rats in Phasic and Tonic Nociceptive Tests

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McLAUGHLIN, C. R. AND W. L. DEWEY. *A comparison of the antinociceptive effects of opioid agonists in neonatal and adult rats in phasic and tonic nociceptive tests.* PHARMACOL BIOCHEM BEHAV **49**(4) 1017-1023, 1994. – Changes in the attitudes about neonatal pain and pain management have recently resulted in increases in the administration of opioids to neonates. Little is known, however, about the relative potencies of the various opioid agonists employed, especially in comparison to adult responses. The first objective in the present study was to compare the antinociceptive potency of four clinically relevant opioids in neonatal and adult rats. The second objective was to compare and contrast these agents in two different types of nociceptive tests: tonic (formalin-induced inflamation) and phasic (tail flick and hot plate). Our results indicate that the opioid agonists morphine, meperidine, and fentanyl, and the mixed agonist buprenorphine were all effective antinociceptive agents in both neonates and adults in each of the three tests employed, and that the relative potencies of these agents appeared to be similar in neonates and adults. In general, the pups were more sensitive to the antinociceptive agents when tested in the phasic nociceptive tests, and the drugs were more potent in the tonic test than either of the phasic tests.

Neonate Tonic nociception Buprenorphine Morphine Fentanyl Meperidine

ATTITUDES and practices in the areas of infant pain and pain management appear to be rapidly changing (18,29). One result of these changes is that analgesic agents are now being used in neonates with increasing frequency but their potency and efficacy have not been thoroughly evaluated in either human subjects or laboratory animals. In a recent survey of board-certified neonatologists in the United States, almost all of the respondents believed that even the youngest and most premature infants are capable of perceiving pain, and most indicated that they used anesthetic and analgesic agents when appropriate (29). This is in marked contrast to earlier reports that highlighted large discrepancies in medicating practices for infants and adults undergoing similar medical procedures, especially when opioids were employed (7,32,34,42). Recent evidence indicates, however, that human infants not receiving appropriate pain management mount a robust physiological stress response that increases both mortality and morbidity (4,5). The judicious use of anesthetic and analgesic agents has been shown to attenuate this stress response, thereby attenuating some of the resultant damage (5,6,22).

Several clinical studies outline the use of various anesthetic and analgesic agents in infants; however, these studies are often reports of the authors' own experiences with various drug preparations and include single case reports. Although it is clear that opioids can serve as effective analgesics in newborns [for review, see (38,43)], it is difficult to draw general conclusions from these studies. Direct comparisons between drugs and across ages are often not feasible due to the lack of standard assessment techniques, dependent measures, and ethical concerns over the use of human subjects. In contrast, it is well known that opioids can produce robust antinociception in neonatal rats in a variety of nociceptive tests including the tail flick, hot plate, and formalin test of tonic nociception (16,17,20,24,30,35). No direct ontological comparisons have been made, however, with several drugs or differing nociceptive tests. Therefore, the primary objective of the present study was to compare the dose responsivity of neonatal and adult rats to three commonly used opioid agonists (morphine sulfate, meperidine hydrochloride, and fentanyl citrate) and one mixed agonist/antagonist (buprenorphine hydrochloride).

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Nociception was once thought to be mediated by one specific neural system. Dennis and Melzack (13), however, suggest that nociception may not be subserved by one single mechanism, but may, instead, be mediated by differing anatomical or biochemical substrates. They have postulated that these differing mechanisms are dependent upon the temporal characteristics of the nociceptive stimulus and the extent to which it damages the tissue. The first type of nociception, phasic nociception, is a brief transient pain present during the first few moments following injury, or elicited by a brief noxious stimulus. Phasic nociception is thought to be subserved by well-myelinated rapidly conducting neural pathways, and is that which is most commonly elicited in the study of nociception in animals (13). The response to this type of nociception is usually an escape or withdrawal response, and two standard tests of phasic nociception are the tail flick (12) and hot plate tests (41). The second type of nociception, tonic nociception, is a continuous pain that usually results from pathology, trauma, or disease, and may have a strong affective-motivational component. Dennis and Melzack (13) have proposed that tonic nociception is subserved by more slowly conducting unmyelinated pathways, resulting in a recuperative response after the injury has been sustained. The formalin test provides a quantifiable measure of tonic nociception in unrestrained animals [for a review of the method, see (37)]. It involves the injection of a small volume of dilute formalin solution into the dorsal surface of a hindpaw. Researchers who have experienced the test themselves have described the pain as initially burning that gradually subsides and is replaced by a dull ache most likely resulting from the inflammation (3,21). Neonatal and adult rats respond to the formalin stimulus with the stereotyped recuperative responses of pawlicking and pawlifting (21,30). Therefore, a second objective of this study was to compare and contrast the potency of these opioids in two different types of nociceptive tests, tonic and phasic, in the two different ages of rat, neonate and adult.

METHOD

Animals

Male Sprague-Dawley rats weighing approximately 250 g (Harlan Sprague-Dawley, Indianapolis, IN) and 3-day-old rat pups of both sexes, derived from Harlan Sprague-Dawley breeding pairs and bred in our colony, were used. Approximately 2 weeks prior to parturition, the dams were individually housed in plastic tub cages until the conclusion of the study. The dams were checked daily for pups with the day of birth designated as day 0. The colony was maintained on a 12 L : 12 D cycle at approximately 22°C with food (Prolab, Agway) and water available ad lib. The litters employed in this study were not culled; however, litters containing less than eight pups were not used. All adult animals were handled and exposed to the testing environment for several days prior to the start of the experiment in an effort to decrease the stress associated with nociceptive testing.

Drugs

A minimum of six animals per dose were employed in each experiment and the observer was blind to drug condition in all experiments. All drugs were obtained from the National Institute on Drug Abuse (Rockville, MD), and are expressed as either milligrams or micrograms of the salt. All drugs were administered intraperitoneally (IP). To ensure that the peak antinociceptive action of the drug coincided with the nociceptive test, morphine sulfate and buprenorphine hydrochloride were administered 30 min before the start of testing, meperidine hydrochloride was administered 10 min before the start of testing, and fentanyl citrate 5 min before the start of testing for the adults as well as the pups. These times were empirically derived based upon earlier reports in the literature (15,30) and pilot studies. To prevent hypothermia, the pups were placed on a heating pad set to approximately 37°C. All pups were euthanized immediately after testing with 0.2 ml sodium pentobarbital (50 mg/ml, IP).

Nociceptive Tests

Formalin. A small volume (5 μl in pups, 50 μl in adults) of dilute formalin (15%) was injected subcutaneously into the dorsal surface of a hindpaw (30). Behavior was time sampled and recorded at 30-s intervals 30–40 min following the formalin injection (30). This time period corresponds to peak nociceptive responding to the formalin-induced inflammation for both adults (15) and pups (30). The antinociceptive agents were administered at the appropriate times after the formalin injection such that the peak drug effect corresponded to the peak formalin response. Nociceptive behaviors were categorized as: pawlift or pawlick. All other behaviors were scored as other. This method of neonatal formalin test was selected because the concentration of formalin and the behavioral ratings were the same for both the pups and adults, thereby allowing direct comparison (30).

Hot plate and tail flick. The animals were placed on a standard tail flick apparatus and the latency to tail flick was automatically recorded (12). The intensity of the heat stimulus was adjusted to yield baseline latencies of approximately 2–3 s for the adults and 4 s for the pups (16). An automatic cutoff of 10 s was employed to prevent tissue damage. Immediately after the tail flick test, the animals were evaluated in the hot plate test of nociception (41). The adult animals were gently placed on the surface of the hot plate (51.5 ± 0.5°C; IITC, Inc., MOD 35-D). Latency to lick a hindpaw, to the nearest 0.1 s, was the dependent measure. Plexiglas walls (45 cm high) surrounded the hot plate during testing of the adult animals to prevent escape. Due to their rather limited motoric capabilities, the pups were held slightly above the hot plate (51.5 ± 0.5°C) with one hindpaw touching the surface. Latency to lift the hindpaw, to the nearest 0.1 s, was recorded (17). Animals not making the appropriate response before the cutoff (60 s for adults, and 15 s for the pups) were removed from the hot plate to prevent tissue damage, and given the maximum score. Three hot plate baselines were taken, and one and two tail flick baselines were collected from the pups and adults, respectively. After the last baseline was taken, the animals were injected with drug. At the appropriate time after injection, antinociception was assessed in the tail flick and hot plate tests as described above.

Statistical Analysis

For the purpose of analyzing the formalin data, paw licking and paw lifting were operationally pooled into a single nociceptive behavior score. The percentage of time engaged in nociceptive behavior was analyzed by two-way analysis of variance (ANOVA) with dose and age as factors, and the Scheffe post hoc analysis was conducted when appropriate. For the purpose of calculating the analgesic dose 50% (AD50), the data were transformed to quantal data (1,30). Animals exhibiting antinociceptive behavior 90% of the time or more were classified as analgesic. The AD50s and 95% confidence

limits (CL) were calculated from these normalized data using the Litchfield Wilcoxin test (27).

Tail flick and hot plate latencies were transformed to the percentage of the maximum possibe effect (MPE) (14) by the following equation:

$$\%\ \text{MPE} = [(\text{test latency} - \text{control latency})/(\text{cutoff criterion} - \text{control latency})] \times 100;$$

where the control latency was the final baseline latency.

The tail flick and hot plate data were also analyzed using two-way ANOVA with dose and age again being the two factors. AD50s and 95% CLs were generated from the linear portion of the dose-response curve using the method described by Tallarida and Murray (36). All potency ratios were calculated using the method described by Tallarida and Murray (36).

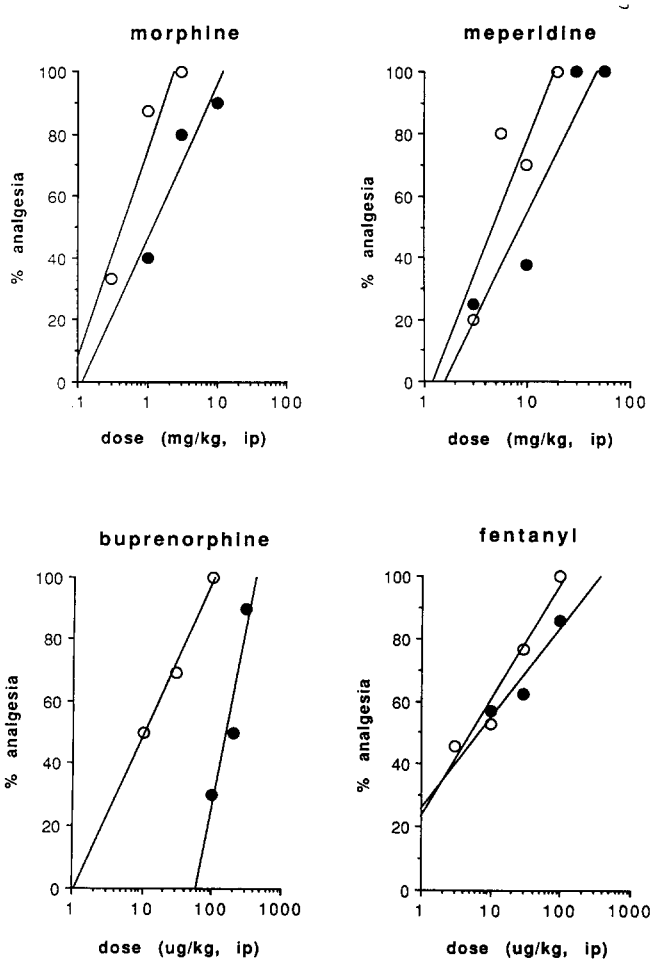

FIG. 1. Dose-response relationship of the antinociceptive effects of morphine, meperidine, buprenorphine, and fentanyl in neonatal (○) and adult (●) rats in the formalin test of tonic nociception. Briefly, a small volume (5 μl in pups, 50 μl in adults) of dilute formalin (15%) was injected subcutaneously into the dorsal surface of a hindpaw (30). Behavior was time sampled and recorded at 30-s intervals during the period of peak nociceptive responding, 30–40 min following the formalin injection (30). All antinociceptive agents were injected after the formalin injection such that the peak drug effect corresponded to the peak formalin response.

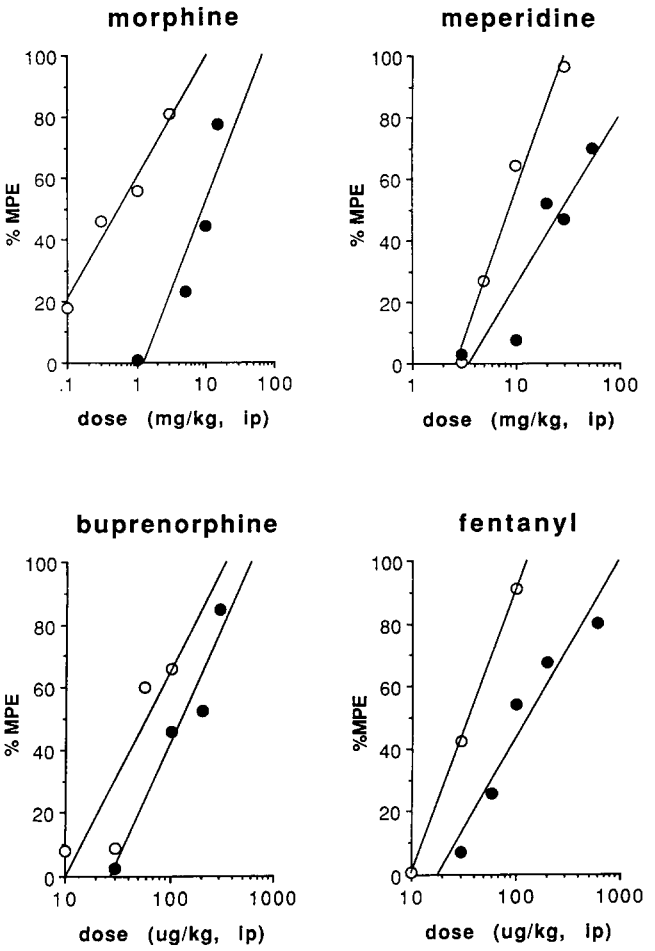

FIG. 2. Dose-response relationship of the antinociceptive effects of morphine, meperidine, buprenorphine, and fentanyl in neonatal (○) and adult (●) rats in the tail flick nociceptive test. Baseline latencies were approximately 2–3 s for the adults and 4 s for the pups (16). An automatic cutoff of 10 s was employed to prevent tissue damage.

RESULTS

Formalin

The results of the formalin test are depicted in Fig. 1. The amount of nociceptive responding during the 10-min test did not significantly differ between vehicle-treated adults and pups (Scheffe's test, $p > 0.05$). Buprenorphine was significantly more potent in the neonates than adults, as indicated by significant main effect of age, $F(1, 81) = 6.02$, $p < 0.05$. In contrast, morphine, meperidine,and fentanyl were all equipotent in the neonates and the adults as indicated by an insignificant main effect of age [morphine, $F(1, 66) = 1.60$, $p > 0.05$; meperidine, $F(1, 80) = 0.026$, $p > 0.05$; fentanyl, $F(3, 89) = 1.95$, $p > 0.05$]. The fentanyl results are interesting, in light of the apparent increased sensitivity in the neonates to the cardiovascular depressive effects of fentanyl reported below.

Tail Flick

The results of the tail flick test are shown in Fig. 2. Significant main effects of age for morphine, meperidine, and fen-

tanyl indicated that they were more potent in the neonates than adults in the tail flick test [morphine, $F(1, 81) = 5.38, p < 0.05$; meperidine, $F(1, 112) = 7.75, p < 0.01$; fentanyl, $F(1, 82) = 4.03, p < 0.05$]. There was no significant difference between neonates and adults in buprenorphine-induced antinociception as indicated by an insignificant main effect of age [age, $F(1, 109) = 0.91, p > 0.05$].

Hot Plate

The hot plate results are depicted in Fig. 3. In the hot plate test, significant main effects of age were found with all of the drugs tested, indicating that they were all significantly more potent in the neonates than in the adults [fentanyl, $F(1, 82) = 6.24, p < 0.05$; buprenorphine, $F(1, 109) = 23.03, p < 0.001$; morphine, $F(1, 81) = 7.40, p < 0.01$; meperidine, $F(1, 112) = 18.61, p < 0.001$].

Ontological Comparison of Opioid Potencies

The AD50s, 95% CLs, and potency ratios between pups and adults are summarized in Table 1. All drugs tested were effective antinociceptive agents in the pups as well as the adults, producing dose-related decreases in nociceptive responding. The relative potencies of the drugs tested were similar in both adults and pups (fentanyl > buprenorphine > morphine > meperidine) (Table 1). Due to differences in the stimulus intensity between the pups and adults in the tail flick test, a morphine dose-response curve was repeated in the adults under conditions similar to those employed in the pups (4 s baseline). Adjusting the stimulus intensity in the adult animals, however, did not significantly alter their responsivity to morphine-induced antinociception, and yielded an AD50 that remained significantly different than that obtained for the pups (adult AD50, 4 s baseline = 5.80 mg/kg, 95% CL = 1.44-23.75; pup AD50, 4 s baseline = 0.54 mg/kg, 95% CL = 0.24-1.21). An examination of the potency ratios between all three nociceptive tests revealed that the drugs were generally equipotent in the tail flick and hot plate tests, and that in most cases the formalin test was more sensitive to the antinociceptive properties of the drugs tested in both neonates and adults (Table 2). An exception to these trends was buprenorphine in the adults, which was almost twice as potent in the tail flick than the hot plate test, and was equipotent in the tail flick and hot plate tests when compared to the formalin test. The potency ratios for both hot plate and tail flick when compared to the formalin test also tended to be larger in the adults, again with the exception of buprenorphine where the ratios were larger for the pups.

Finally, all of the pups ($n = 3$) treated with high doses of fentanyl (300 μg/kg, fentanyl) and meperidine (56 mg/kg, meperidine) died (data not shown), while no lethality was observed in the adults.

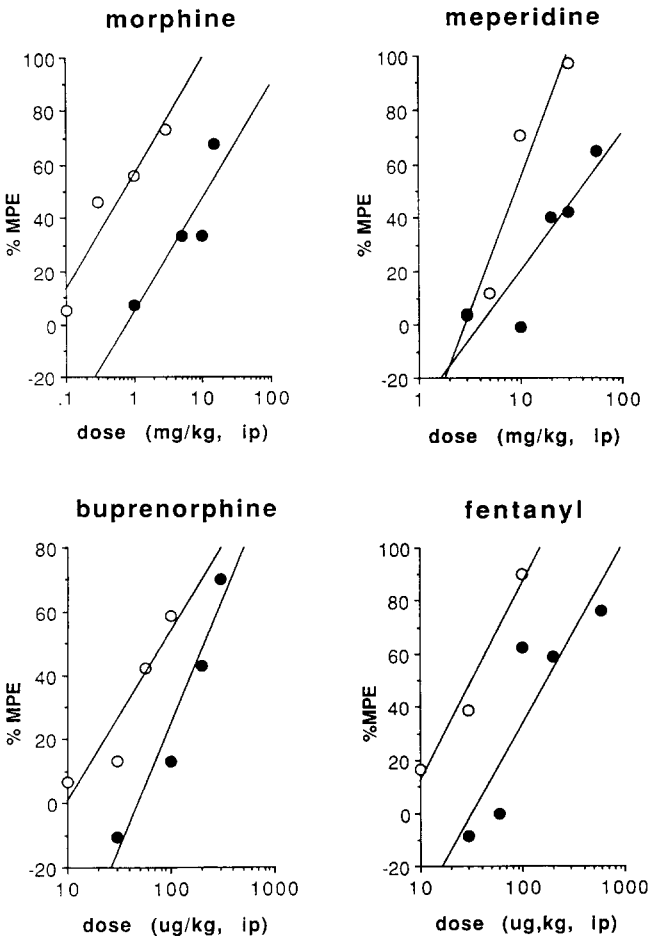

FIG. 3. Dose-response relationship of the antinociceptive effects of morphine, meperidine, buprenorphine, and fentanyl in neonatal (○) and adult (●) rats in the hot plate (51.5 ± 0.5°C) nociceptive test. Animals not exhibiting a nociceptive response before the cutoff (60 s for adults, and 15 s for the pups) were removed from the hot plate to prevent tissue damage and given the maximum score.

DISCUSSION

The primary aim of this study was to compare and contrast the potencies of three frequently used opioid agonists and one mixed opioid agonist/antagonist in both neonatal and adult rats in three different nociceptive tests. Our results indicate that the opioid agonists morphine, meperidine, and fentanyl, and the mixed agonist/antagonist buprenorphine were all effective antinociceptive agents in neonates in the three tests employed. Furthermore, the rank order of potencies of these agents in pups was similar to those observed in adults. Contrary to previous reports (31,44), opioids were not less potent antinociceptive agents in neonates when compared to adults. In fact, the pups were consistently more sensitive than the adults to all drugs evaluated in the hot plate test. Morphine, meperidine, and fentanyl were also more potent in the pups in the tail flick test, while buprenorphine was found to be equipotent in neonates and adults in the same test. On the other hand, only buprenorphine was found to be significantly more potent in the pups in the formalin test; there were no differences in sensitivity between the pups and adults to morphine, meperidine, or fentanyl. Finally, both fentanyl and meperidine appeared to be more lethal in the pups as they produced death at the highest doses tested. These findings indicate that the pups may be more sensitive to the cardiovascular depression associated with higher doses of fentanyl and meperidine, similar to that previously reported for morphine (19,25,31,40,44).

The second objective of the present study was to compare and contrast these antinociceptive agents in two different types of nociceptive tests, phasic and tonic, which earlier data indicate are different biochemically and neuranatomically. In

TABLE 1

COMPARISON OF THE ANTINOCICEPTIVE POTENCIES* OF OPIOID AGONISTS IN NEONATAL AND ADULT RATS

	Adult	Pup	Potency Ratio (Pup : Adult)
	Formalin Test		
Fentanyl	0.008 (0.002–0.037)	0.006 (0.002–0.012)	1.8
Buprenorphine	0.154 (0.104–0.230)	0.012 (0.005–0.025)	12.2
Morphine	1.21 (0.48–3.07)	0.42 (0.21–0.81)	3.7
Meperidine	9.02 (4.29–18.95)	4.71 (3.03–7.34)	2.1
	Tail Flick Test		
Fentanyl	0.140 (0.074–.265)	0.040 (0.009–0.172)	3.5
Buprenorphine	0.139 (0.074–0.263)	0.065 (0.012–0.346)	2.3
Morphine	9.23 (5.17–16.47)	0.54 (0.24–1.21)	20.4
Meperidine	29.99 (15.05–59.75)	9.27 (4.95–17.37)	2.7
	Hot Plate Test		
Fentanyl	0.211 (0.020–2.265)	0.031 (0.003–0.290)	5.3
Buprenorphine	0.217 (0.147–0.319)	0.083 (0.028–0.251)	3.3
Morphine	10.95 (2.88–41.65)	0.82 (0.15–4.46)	16.0
Meperidine	33.57 (22.06–51.10)	8.83 (5.85–13.33)	3.3

*Analgesic dose 50% (AD50), mg/kg, IP (95% confidence limits).

general, the drugs were more potent in the formalin test of tonic nociception than in either the tail flick or hot plate test, with the exception of buprenorphine in the adults, which was equipotent in the formalin test when compared to the tail flick and hot plate tests. This observed increase in potency in the tonic nociceptive test was especially true for the adults where the potency ratios were generally higher.

Two explanations exist for our finding of differential sensitivity between the tonic and phasic nociceptive tests. First, the formalin test has been reported to be mediated by differing anatomical and biochemical mechanisms. Previous studies have demonstrated the differential potency of morphine, enkephalinase inhibitors, and the kappa agonist PD 117302 when tonic and phasic nociceptive tests were compared directly (1,10,11). Furthermore, several lines of research support the anatomical distinction of tonic and phasic nociception suggested by Dennis and Melzack (13). In a study by Abbott and colleagues (2), it was shown that lesions of the caudal periaqueductal gray and the nucleus raphe magnus (NRM) attenuated morphine-induced antinociception in a phasic nociceptive test but not a tonic test. The anatomical differences between tonic and phasic nociception are further exemplified by electrolytic lesions of the dorsal longitudinal funiculus (DLF) of the spinal cord, as lesions of the DLF attenuate morphine-induced antinociception in a phasic but not tonic nociceptive test [see review in (33)]. Cohen and Melzack (9) have also produced naloxone-reversible analgesia in the formalin test with discrete microinjections of morphine into the habenula and nucleus dorsalis posteromedialis of the thalamus (H-PMT). Microinjection into structures immediately adjacent to the habenula and the H-PMT, however, produced no analgesia highlighting a regional specificity. Vaccarino and Melzack (39) have also shown that reversible lesions of the cingulum, a structure with connections to the thalamus as well as other cortical and limbic structures, attenuates formalin-induced nociception but not phasic nociception. These data again lend support to the hypothesis that tonic and phasic nociception are subserved by anatomically distinct loci. Finally, Matthies and Franklin (28) have recently shown that chronic decerebration interferes with morphine-induced anti-

TABLE 2

POTENCY RATIOS AMONG THE NOCICEPTIVE TESTS FOR PUPS AND ADULTS

		Formalin : Tail Flick	Formalin : Hot Plate	Tail Flick : Hot Plate
Fentanyl	Pup	3.4	3.1	0.9
	Adult	11.1	17.7	1.3
Buprenorphine	Pup	6.2	8.0	1.2
	Adult	0.9	1.4	1.7
Morphine	Pup	2.1	2.7	1.3
	Adult	8.0	8.4	1.0
Meperidine	Pup	1.8	1.8	1.0
	Adult	3.5	4.5	1.0

nociception in the formalin but not the tail flick test, suggesting an important role for supraspinal mediation of morphine-induced antinociception in the formalin test of tonic nociception.

Alternatively, the recuperative response elicited in the formalin test involves a very complex and highly integrated motor task that may be more sensitive to motor impairment than the phasic nociceptive tests. However, both the hot plate and formalin tests require essentially the same behavior, lifting and licking a hindpaw. However, the hot plate test showed a dose responsivity to the antinociceptive agents that was comparable to the tail flick test, a test that is relatively insensitive to motor impairment. Therefore, it is unlikely that motor impairment would account for the differential responsivity observed between the tonic and phasic nociceptive tests.

The data in the present study are consistent with previous reports that revealed robust morphine-induced antinociception in neonates in the tail flick, hot plate, and formalin tests (16,17,20,24,30,35). Our data are also consistent with earlier findings in both humans and rats that indicated an increased sensitivity to morphine-induced respiratory depression in neonates when compared to adults (19,25,40). After extensive study, Kupferberg and Way (25) concluded that an increased permeability of the blood–brain barrier in neonates may mediate this heightened sensitivity to morphine-induced respiratory depression (25). This postulated increase in blood–brain barrier permeability to opioids may also mediate the increased sensitivity to the antinociceptive properties of the drugs observed in the present study.

It is important to note, however, that the data in the present study also stand in contrast to previous reports that indicated marked insensitivity to the antinociceptive properties of morphine in 2-day-old rat pups (31,44). These authors have suggested that the development of antinociception parallels the postnatal ontogeny of the opioid receptors when mu binding is normalized as a function of wet tissue weight (31,44). However, when mu binding is assessed as a function of protein rather than wet tissue weight, it appears that receptor number [for review see (26)] and receptor affinity (8) are not significantly lower in neonates when compared to adults. In addition, one important methodological difference between these reports that indicate a developmental delay in opioid-mediated antinociception and the present study and others which report antinociception early in development (16,35) is the intensity of the tail flick stimulus. The tail flick stimulus in studies reporting no significant antinociception in neonates was presumably very intense and was not adjusted to account for the pups' thinner tail skin; studies reporting antinociception in neonates use a lower intensity tail flick stimulus to account for these anatomical differences. It has been demonstrated previously that large differences in stimulus intensity significantly decrease the potency and efficacy of opioid antinociceptive agents (23), and pilot data obtained in our laboratory confirm that significant increases in the intensity of the tail flick stimulus, resulting in baselines similar to those reported in the earlier studies (31,44), completely obliterated any morphine-induced antinociception. Despite the fact that the stimulus intensity was decreased somewhat for the neonates in the tail flick test, however, the results were not significantly different from those obtained in the hot plate test where no adjustment in stimulus intensity was made. Finally, as we have demonstrated, increasing the baseline latency from 2–3 s to 4 s in the adult animals did not alter their responsivity to morphine-induced antinociception. In summary, these findings indicate that the differences observed between the neonates and adults in the tail flick test were due to increased sensitivity in the neonates to the agents tested, and not differences in stimulus intensity.

In conclusion, attitudes about pain and pain management in infants appear to be rapidly changing, and aggressive pain management, especially with opioids, is now being reported in even the youngest patients (29,32,42). Although there exist a number of isolated studies examining single pharmacological agents, there have been no studies employing a constant and readily quantifiable dependent measure of pain at different ages such that accurate comparisons could be made about the potency of various commonly employed analgesic agents (29). Therefore, in the present study we evaluated the potency of three opioid agonists and one mixed agonist/antagonists in three commonly used nociceptive tests, and found all agents to be effective antinociceptive agents in all three tests. The relative potencies of the drugs tested were similar in adults and pups in all tests, indicating that they respond to these agents in a qualitatively similar fashion. In addition, the drugs were generally more potent in the neonates in the tail flick and hot plate tests, suggesting that analgesic agents effective in humans are likely to be more potent in infants. Finally, the data from the present study indicate that the drugs were equipotent in the tail flick and hot plate tests, yet more potent in the formalin test of tonic nociception. These findings are consistent with earlier data that indicate that phasic and tonic nociceptive tests differ biochemically and neuranatomically in their responsivity to antinociceptive preparations.

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