

Adverse Effects of Paternal Opiate Exposure on Offspring Development and Sensitivity to Morphine-Induced Analgesia¹

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ABSTRACT

We have shown previously that chronic morphine administration to adolescent male rats produced a number of gender specific deficits in their offspring. The purpose of the present studies was to extend our earlier observations by examining the acute, direct effects of morphine exposure to male rats on their fertility and the development of viable offspring. Sexually mature male rats were injected with a single dose of morphine (25 mg/kg) and 24 hr later were bred with drug-naive females. Fertility rates (vaginal plugs and pregnancies) were monitored throughout the breeding period as was the development of the offspring. Our results showed that a large, acute dose of morphine given to drug-naive male rats 24 hr before the initiation of breeding had no effect on fertility rates, but produced several adverse effects on fetal outcome. Litter sizes in morphine-

derived offspring were considerably smaller than in controls and mortality rates were more than 6 times higher. Moreover, morphine-derived male, but not female, offspring had a significantly enhanced sensitivity to the antinociceptive effects of morphine. Collectively, these data suggest that acute paternal morphine exposure just before breeding with drug-naive females had no effect on fertility, but exerted negative effects on the viability and development of their offspring. These results represent the most compelling evidence to date that paternal opiate exposure can adversely affect fetal outcome and are particularly striking in that they were produced by a single injection of morphine. We are aware of no animal studies, clinical cases or anecdotal reports in humans in which such a phenomenon has been described.

We have shown previously that chronic morphine administration to adolescent male rats significantly retarded the onset of puberty and sexual maturation (Cicero *et al.*, 1989, 1991a). As an extension of these studies, we bred morphine-exposed males with drug-naive females to determine whether there were residual effects of adolescent opiate exposure, 2 to 3 weeks after drug withdrawal, on male copulatory behavior and the production of viable offspring. We observed normal mating behavior and the same number of births in drug-naive females mated with morphine-exposed males when compared to controls. Moreover, with the exception of smaller litter sizes, the offspring of morphine-exposed fathers appeared to develop normally. However, prompted by several reports in the literature that paternal exposure to environmental toxicants or drugs produced often subtle adverse effects on their offspring (Brady *et al.*, 1975; Friedler, 1975, 1985; Cohen, 1986; Friedler and Cicero, 1988; Abel, 1992, 1993), we explored the possibility that the offspring of morphine-exposed fathers had deficits as adults which were

not readily detectable upon gross observation. We found a number of selective gender-specific endocrine differences in morphine-derived offspring when compared to controls. For example, adult male offspring of morphine-treated males had significantly lower serum testosterone and luteinizing hormone levels than male offspring of saline-treated males. A somewhat different pattern of endocrine deficits was observed in adult female morphine-derived offspring. Large increases in serum corticosterone levels were observed in female morphine-derived offspring and, in addition, β -endorphin levels in the hypothalamus also were significantly higher than in controls. In contrast to the male, no differences in reproductive endocrine status were observed in adult female morphine-derived offspring. Thus, our prior data suggest that a period of exposure of males to opiates during adolescence can have long-term effects on their capacity to conceive normal offspring, particularly in terms of selective, gender specific deficits in endocrine function.

Our earlier studies were limited in two important respects. First, the range of potential deficits examined was restricted and the biological significance of many of our findings was unknown. Second, in our earlier studies we used chronic morphine administration for prolonged periods of time (6 weeks or more). Hence, it is uncertain whether the effects observed were direct effects of morphine on the male's repro-

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ABBREVIATIONS: HPA, hypothalamic-pituitary-adrenal; RIA, radioimmunoassay; % MPE, percentage of maximal effect.

ductive capacity or rather resulted from some nonspecific confounding variable associated with chronic drug administration, such as stress, organ dysfunction or poor nutrition, any of which can profoundly affect reproductive status in the male (Adler and Toner, 1986; Rivier and Rivest, 1991). Thus, we felt that an analysis of the acute, direct effects of morphine on fertility and the development of their offspring would be a more appropriate model to manipulate pharmacological variables, control for nondrug related confounds and carry out assessments of the mechanisms involved in the paternal effects of opiates.

To accomplish the aforementioned goals, sexually mature male rats were injected with a single dose of morphine (25 mg/kg). Twenty-four hours later they were bred with drug-naive females. Fertility rates (vaginal plugs and pregnancies) were monitored as was the development of their offspring. In order to determine whether any effects of paternal morphine exposure were specific to this drug, two additional groups were included: male rats injected with cocaine or stressed for 1 hr in a restraint device 24 hr before breeding with drug-naive females. The rationale for using these additional groups was: first, to determine whether other commonly abused drugs, such as cocaine, produced effects similar to those observed with morphine; and, second, to examine whether activation of the HPA axis produced either by cocaine or restraint stress produced effects similar to morphine. Whereas the rationale for the first objective seems clear, the purpose for assessing the role of stress may be less obvious. Morphine, cocaine and, of course, restraint stress produce robust increases in the activity of the HPA axis (Kalra and Kalra, 1983; Cicero, 1987; Rivier and Rivest, 1991; Cicero and Adams, 1995). Given the well known role of stress in affecting mating behavior and reproductive function in the male rat (Rivier and Rivest, 1991), it was important to validate that any effects observed with morphine were specific drug effects as opposed to a confound related to stress or activation of the HPA axis.

Materials and Methods

Animal and chemicals. All animals used in these studies were bred in our colony from a stock supplied by Harlan Sprague-Dawley, Inc. (Cumberland, IN). Animals were bred no more than twice before they were replaced to avoid excessive inbreeding. The desirability of using animals bred in our colony, as opposed to purchasing them from commercial sources, was to avoid the stress of shipping which we have found to be a significant confounding variable in drug-endocrine interaction studies. Morphine sulfate was generously provided by the National Institute on Drug Abuse (Rockville, MD) and cocaine-HCl was purchased from Sigma Chemical Co. (St. Louis, MO). The iodinated morphine RIA kit was purchased from Diagnostic Products Corporation (Los Angeles, CA).

Drug treatment and breeding protocol. Male adult rats (60–75 days of age) were injected i.p. with morphine (25 mg/kg) or cocaine (40 mg/kg divided into two doses of 20 mg/kg given at 9:00 A.M. and 4:00 P.M.). An additional experimental group was restrained in disposable rodent restraining cones (DecapiCones, Brain Tree Scientific, Brain Tree, MA) for 1 hr. Two control groups also were utilized: one was injected with saline (to control for the irritation caused by the injection of morphine or cocaine), whereas the second was left undisturbed (to control for both drug injections and restraint stress). Twenty-four hours after the respective treatments, males were housed individually with drug-naive adult females (90–120 days of age) who were either nulliparous or primiparous. These two

groups of females were used to determine whether prior sexual history and maternal behavior needed to be accommodated as an experimental variable.

The males and females were housed together until a vaginal plug was detected or 21 days had elapsed, whichever occurred first. Vaginal plugs were checked daily (9:00 A.M.) and the number of births by day were recorded. At birth, the litters were left undisturbed and were culled to eight pups (four males and four females) at 7- to 8-days of age. The animals were weaned at 22 days of age and were then used as adults in the experiments described below. At postnatal day 0 (day of birth) 7, 14, 21, 28, 45 and 60, the pups were weighed and mortality rates were recorded. Gross indices of development, such as incisor eruption, testes descent or vaginal opening, appearance of hair and eye opening were examined daily.

Assessment of the antinociceptive activity of morphine. At 60 to 75 days of age, groups of male offspring of morphine-, cocaine- or stress-exposed fathers and the appropriate controls (saline-injected or untreated) were injected with one of two doses of morphine (10 or 12.5 mg/kg). Female offspring were injected with 15 mg/kg. These doses were selected to approximate the ED₄₀ to ED₈₀ for morphine-induced antinociceptive activity on the hot-plate (Cicero and Meyer, 1973). Rats were placed on a hot-plate that was set at 58°C. Typical responses to this thermal pain were paw-licking or jumping out of the cylinder. The test was terminated when either of these responses occurred or if the 30-sec cutoff time elapsed. In all studies, three base-line (*i.e.*, nondrug-treated) trials were run, the animals were then injected with morphine and trials were run every 30 min until response times returned to base-line levels. Data were expressed both in terms of absolute reaction time in seconds or % MPE. The % MPE was calculated according to the following formula:

$$\% \text{ MPE} = \frac{\text{actual response time (sec)} - \text{baseline (sec)}}{30 \text{ sec (cutoff)} - \text{baseline (sec)}} \times 100$$

Blood levels of morphine. To determine the peak morphine levels in blood achieved after a single dose of morphine and, more importantly, whether any residual morphine was present 24 hr after the injection when mating began, adult male rats were injected with the dose of morphine (25 mg/kg) used in the breeding studies. At intervals thereafter, groups were sacrificed for an assessment of blood levels of immunoreactive morphine. Blood levels of morphine were determined by the RIA kit described above.

In an additional series of studies, we examined whether the differences in sensitivity to morphine's antinociceptive effects observed in offspring derived from morphine-exposed fathers might be attributed solely to altered metabolism. Groups of male offspring sired by morphine-exposed or control fathers were injected with morphine at a dose of 12.5 mg/kg, which produced the largest difference between morphine- and control-derived offspring in the antinociceptive studies (see "Results"). At the intervals used in the hot-plate testing, groups of animals were sacrificed and serum was collected for the determination of blood morphine levels. Female offspring were not assessed in these studies, because we found no differences between morphine-derived and control female offspring in the analgesia studies.

Statistical analysis. In all studies of offspring outcome and morphine-induced analgesia, the litters were considered the unit of measure for the experimental design and statistical analysis. Specifically, in those instances in which offspring outcome (*e.g.*, body weights, mortality rates, etc.) were assessed, means were taken for each litter and this litter mean was used as the unit of measure in subsequent analyses. In the antinociceptive testing, each group consisted of animals drawn from separate litters to control for litter effects. All parametric data were analyzed either by *t* tests when only two groups were being compared or by repeated measure analysis of variance followed by post-hoc analyses. Nonparametric data (*e.g.*, pregnancy and mortality rates) were analyzed using the Chi Square test.

Results

Effects of morphine on fertility and fetal outcome.

Figure 1 shows the fertility parameters in drug-naive females mated with morphine- or saline-treated male rats and several fetal outcome measures (litter size and mortality rates). These results represent the combined results of two replicate studies involving a total of 40 mating pairs (*i.e.*, 40 males injected with morphine or saline bred individually with the 40 drug-naive females). There were no differences between the number of vaginal plugs in females mated with morphine or saline-treated males (data not shown). Similarly, the number of live births was equivalent in both groups (fig. 1, left panel). Specifically, 85% of females mated with saline-treated males gave birth when compared to 80% of those mated with morphine-treated males. We found no differences between nulliparous or primiparous females on fertility measures and, most importantly, fetal outcome measures and, hence, these data were pooled. In view of the fact that maternal history did not seem to be an important variable in the effects of paternal drug treatment, nulliparous females were used in all subsequent studies described in this paper.

Despite the data showing apparently normal fertility parameters in morphine-treated males mated with drug-naive females, we found significant effects of paternal morphine exposure on fetal outcome. Litter sizes were significantly smaller (fig. 1, middle panel) and mortality rates were more than 6 times higher in morphine-derived offspring than in controls (fig. 1, right panel; table 1). As shown in table 1, the increase in mortality in morphine-derived offspring was reflected not only in total mortality rates (*i.e.*, percentage of total morphine-derived pups dead), but in the number of litters in which death occurred. We found that deaths occurred in nearly one-half of the morphine-derived litters when compared to 5 to 10% for all other treatment or control groups.

We were unable to detect any other gross developmental anomalies in the offspring of treated fathers utilizing several key indices of development including sex ratios, incisor eruption, testes descent, vaginal opening, eye opening or appearance of hair. Body weights during development were slightly

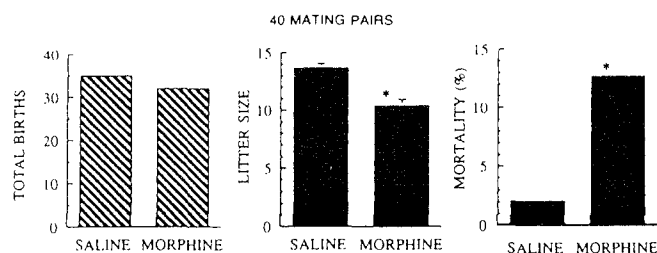


Fig. 1. Total births, litter sizes and litter mortality rates in drug-naive females bred with morphine- or saline-treated males. The data are the combined results from two replicate experiments for a total of 40 mating pairs consisting of drug-naive females bred with males treated with morphine or saline. Forty males were injected with morphine (25 mg/kg) or saline and 24 hr later were housed individually with drug-naive females. Total births represent the number of females that gave birth out of 40 in each group. Values for litter sizes are means (\pm S.E.M.). Mortality rates are expressed as percentage of mortality in the total number of offspring (see table 1 for details). *Significantly ($P < .01$) different than controls.

but not significantly higher in morphine-derived offspring than in controls until they were culled (8-days of age) which is consistent with the somewhat smaller litter sizes observed in these groups. After the litters were culled to eight animals in all groups, there were no significant differences between groups (table 1).

Table 1 summarizes all of the data concerning maternal fertility rates and litter developmental outcomes for all treatment groups (morphine, cocaine, stress and the respective controls) utilized in these studies.

Serum morphine levels in males. As shown in figure 2, after an injection of 25 mg/kg of morphine (the dose administered to males 24 hr before breeding in the preceding experiments), blood morphine levels peaked within 1 hr of morphine administration. Thereafter, blood levels declined rapidly such that by the time mating occurred 24 hr after the injection, morphine was nondetectable in blood.

Effects of cocaine and stress on fertility. As was the case with morphine, pretreatment of male rats either with cocaine or 1 hr of restraint stress resulted in no differences in vaginal plugs or live births as shown in table 1. In marked contrast to our results with morphine, however, we found no differences in litter sizes or mortality rates in the offspring sired by cocaine- or stress-exposed males (table 1). Thus, these data suggest that neither morphine, cocaine or stress affect fertility parameters in males subsequently mated with drug-naive females. More importantly, they indicate that paternal morphine-exposure adversely affects several fetal outcome measures, but that neither paternal cocaine nor stress exposure produces any detectable offspring anomalies.

Sensitivity to morphine's antinociceptive effects in morphine-derived offspring. The results of the hot-plate test in morphine-derived and control male offspring are shown in figure 3. These data represent the results of three separate experiments involving a total of 36 animals drawn from 36 litters in each treatment group. There were no differences in base-line (nondrug treated) reaction times on the hot-plate, suggesting that pain thresholds were equivalent in the two groups. However, we found a dose-dependent enhanced morphine-induced analgesic response in offspring derived from morphine-exposed fathers relative to saline-injected controls. The enhanced analgesic effect of morphine was reflected in the % MPE, peak antinociceptive response which occurred subsequent to morphine (fig. 3), and the duration of analgesia. In figure 3 and all subsequent figures, reaction time in seconds is presented, inasmuch as these data are most informative and are representative of all other means of data presentation (*e.g.*, % MPE and duration of analgesia).

The results with female morphine- and saline-derived offspring are shown in figure 4. There were no differences observed in morphine-induced antinociception between morphine- and saline-derived offspring. The data shown in figure 4 represent the results obtained after an injection of 15 mg/kg of morphine. To determine whether differences between morphine- and saline-derived female offspring might be observed at lower or higher doses, a range of other morphine doses were assessed (2.5–20 mg/kg). The results of these studies confirmed that there were no differences between groups (data not shown).

Sensitivity to morphine's antinociceptive effects in cocaine- and stress-derived offspring. Morphine-induced

TABLE 1
Fertility parameters and fetal outcome measures in treated males mated with drug-naive females

	Male Drug Treatment Conditions				
	Saline	Morphine	Untreated	Cocaine	Stress
Total mating pairs ^a	40	40	20	20	20
Vaginal plugs	37	33	14	16	19
Births	37	34	20	17	20
Litter sizes	13.58 (\pm 0.28)	10.6 (\pm 0.28)*	12.0 (\pm 0.85)	12.05 (\pm 0.78)	12.26 (\pm 0.68)
Male/female ratio	6.45/5.5	5.17/5.4	6.2/5.7	5.5/6.2	5.8/5.7
% Pups mortality ^b	2.03	12.7**	3.33	2.44	1.71
Litter mortality incidence ^c	3/37	16/34**	2/20	2/17	2/20
Pups body weights (birth)	7.98 (\pm 0.66)	9.4 (\pm 1.4)	8.6 (\pm 1.3)	8.4 (\pm 0.81)	8.8 (\pm 0.79)
Pups body weights (weaning)	44.8 (\pm 3.9)	40.3 (\pm 2.4)	42.9 (\pm 2.9)	43.2 (\pm 1.4)	43.3 (\pm 1.6)

^a Mating pairs consisted of one drug-naive female bred with one male injected with either morphine, cocaine or saline or subjected to restraint stress for 1 hr (see "Materials and Methods"). An untreated male control group also was utilized. Males and females were housed together 24 hr after their respective treatments for 21 days or until a vaginal plug was detected, which ever occurred first.

^b Raw data for percentage of mortality are as follows: morphine, 31 of 245 pups (12.7%); saline, 7 of 344 pups (2.03%); untreated, 28 of 240 pups (3.33%); cocaine, 5 of 205 pups (2.44%); stress, 4 of 233 pups (1.7%).

^c Mortality incidence is defined as the number of litters in which pups deaths occurred as a function of the total.

* Significantly ($P < .01$) less than saline-injected controls; ** significantly ($P < .05$) greater than saline-injected controls.

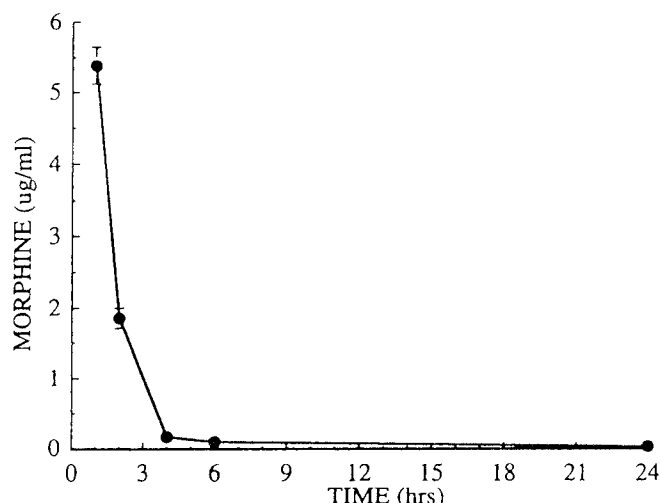


Fig. 2. Mean (\pm S.E.M.) serum morphine levels (micrograms per milliliter) in adult male rats ($n = 10$) injected with 25 mg/kg (at zero time in this figure) and were then sacrificed at the intervals shown.

antinociceptive activity in male offspring derived from cocaine-injected and stressed fathers are shown in figure 5. As can be seen, there were no differences between cocaine- or stress-derived male offspring when compared to controls, after a dose of morphine of either 10 mg/kg (data not shown) or 12.5 mg/kg (fig. 5). As was the case with morphine, we observed no differences between morphine's analgesic activity (over a broad dose range) in female offspring of fathers exposed to cocaine or stress 24 hr before mating.

Serum morphine levels in male offspring. As shown in figure 6, serum morphine levels peaked at 2.5 to 3.0 ng/ml 30 min after the injection of 12.5 mg/kg of morphine (the dose used in our assessment of morphine's antinociceptive effects) in offspring sired by morphine- or saline-treated males. Blood levels then declined rapidly such that they were nondetectable 6 hr postinjection. No differences were observed in the male offspring derived from morphine- or saline-exposed males in blood morphine levels after the injection of morphine either in terms of the peak blood levels attained or in morphine-disappearance curves.

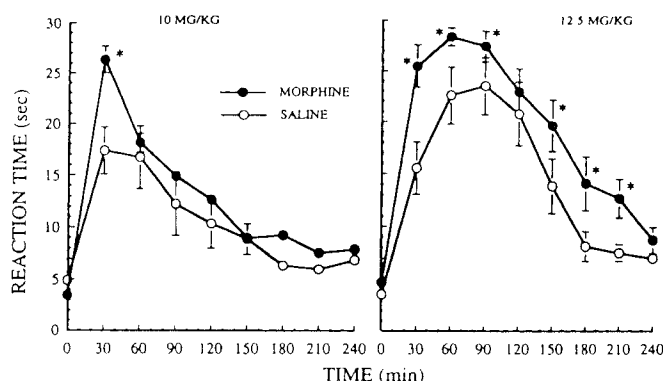


Fig. 3. Mean (\pm S.E.M.) reaction time in morphine- and saline-derived male offspring on the hot-plate test (588C). The values are the aggregate results of three experiments utilizing a total of 36 males (litters) in each group. Morphine, at 10 or 12.5 mg/kg, was injected at zero time. *Significantly ($P < .01$) greater than controls (F values: drug, 5.99, 1,22 dF; time, 35.98, 7,154 dF; drug \times time interaction: 3.79, 7,154 dF. Post-hoc analyses were carried out by using the Neuman-Keuls Method).

Discussion

The results of these studies demonstrate that a large acute dose of morphine given to drug-naive male rats had no effect on fertility parameters (vaginal plugs or pregnancies), but produced several adverse effects on fetal outcome. Litter sizes in morphine-derived offspring were considerably smaller than in controls and mortality rates were markedly higher. Moreover, morphine-derived male but not female offspring had a significantly enhanced sensitivity to the antinociceptive effects of morphine. Collectively, these data suggest that acute morphine administration before breeding with drug-naive females had no effect on copulatory behavior or fertility, but exerted substantial negative effects on the viability and development of their offspring.

The results described in this paper represent the most compelling evidence to date that paternal morphine exposure can adversely affect fetal outcome. These effects are particularly striking in that they were produced by a single injection of morphine. We are unaware of any animal studies, clinical cases or anecdotal reports in humans in which such a

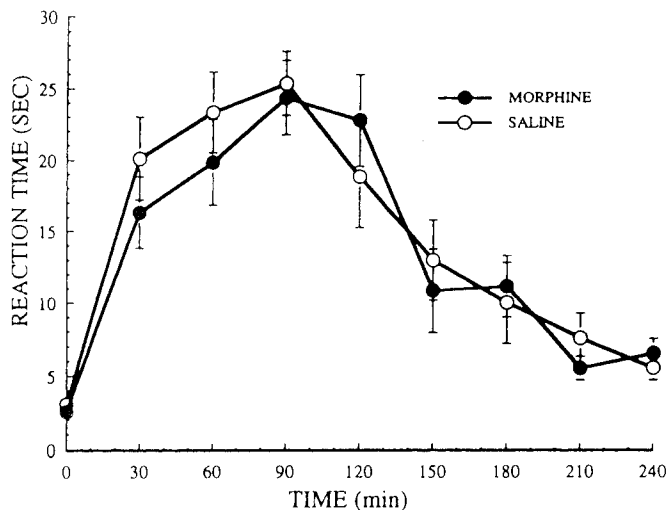


Fig. 4. Mean (\pm S.E.M.) reaction time in morphine- and saline-derived female offspring on the hot-plate test (588C). The values are the pooled results of two experiments utilizing a total of 24 (litters) females in each group. Morphine (15 mg/kg) was injected at zero time.

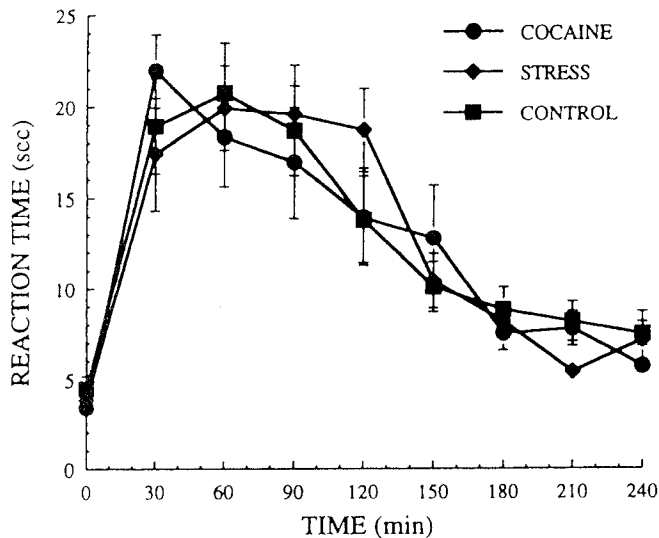


Fig. 5. Mean (\pm S.E.M.) reaction time on the hot-plate (588C) for male control ($n = 24$), cocaine- or stress-derived offspring ($n = 12$ in each experimental group). Data for untreated and saline-injected ($n = 12$ in each group) males were pooled because there were no differences between groups and are therefore labeled control in this figure. Morphine (12.5 mg/kg) was injected at "0" time.

phenomenon has been described. However, several previous studies, including a number from this laboratory, have shown that chronic paternal exposure to opiates (Friedler, 1975, 1985; Friedler and Cicero, 1988; Cicero *et al.*, 1989, 1991a), and other abused substances and toxicants (Brady *et al.*, 1975; Cohen, 1986; Wozniak *et al.*, 1991; Abel, 1992, 1993; Cicero, 1994), can lead to a number of anomalies in their offspring which appear to be selective and often gender specific. The results of many of these chronic studies are, however, difficult to interpret because the confounds associated with chronic drug administration, such as poor nutrition, tolerance and withdrawal phenomena, organ dysfunction and stress, may seriously compromise male reproductive behavior and function (Adler and Toner, 1986; Rivier and Rivest, 1991). Consequently, it has been impossible in these

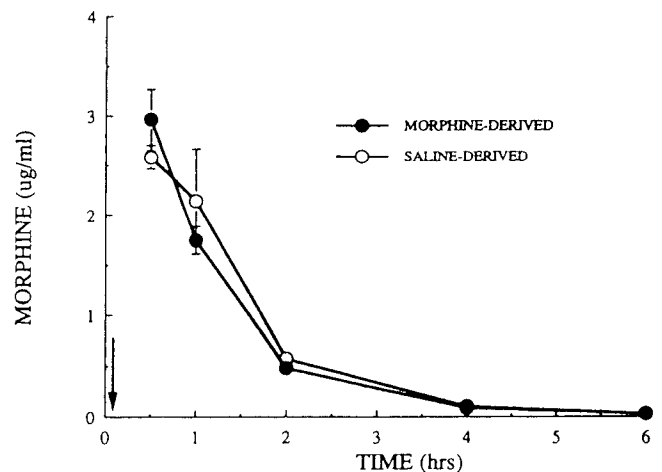


Fig. 6. Mean (\pm S.E.M.) morphine blood levels (microgram per milliliter) subsequent to the injection of 12.5 mg/kg of morphine in morphine- and saline-derived offspring ($n = 10$ at each time point). Morphine was injected at zero time as shown by the arrow in this figure.

studies to determine whether the effects of morphine are drug-specific or represent an epiphenomenon generated by a host of confounding variables. Our studies suggest for the first time that a single exposure of male rats to a large dose of morphine can impair fetal outcome, suggesting that morphine *per se* can induce teratological effects on offspring.

The three most striking effects of paternal morphine exposure on offspring outcome were: smaller litter sizes; markedly enhanced mortality rates; and significantly enhanced sensitivity to morphine's analgesic effects. The mortality rate in morphine-derived offspring was over 6 times higher than that found in controls. Nearly all (>95%) of the deaths occurred from postnatal day 1 through 7. It was impossible, however, to determine the cause of death in these studies inasmuch as the dead or dying pups were almost cannibalized immediately by the mother. The smaller litter sizes observed in litters produced by morphine-injected males can be attributed to one of two mechanisms. First, the smaller number of pups could be due to either pre- or postimplantation loss in the females (*e.g.*, failure of blastocytes to implant in the uterine wall or a higher degree resorption or fetal loss after implantation). Second, it is equally possible that there was significant mortality in litters derived from morphine-treated males within the first several hours of birth. Although litters were checked twice daily for fetal survival rates, it is possible that some fetal loss *via* cannibalization occurred before ascertaining the number of pups in each litter. Studies are currently underway to address whether pre- or postimplantation fetal loss or fetal death within hours of birth can explain the smaller litter sizes observed in females mated with morphine-derived offspring when compared to controls.

In our studies, we also examined whether the sensitivity to morphine was altered in the male and female offspring of morphine-exposed fathers. Our decision to focus on the relative sensitivity to morphine in offspring derived from fathers treated acutely with the opiate before breeding was based on the rapidly accumulating data, which suggest that the children of fathers who abuse drugs, particularly alcohol, have significant cognitive and physiological deficits (Hegedus *et al.*, 1984; Begleiter and Projesz, 1988; Schuckit, 1988; Ehlers

et al., 1989; Tartar *et al.*, 1989; Pickens *et al.*, 1991). One of the more prominent of these deficits is an altered drug sensitivity and a significantly greater risk of developing drug-related problems. These familial studies suggest either that the liability of drug abuse is heritable or, alternatively, that the ingestion of drugs by fathers may induce alterations in their offspring which influence their subsequent sensitivity to the acute and chronic effects of these compounds. In our studies, we found that the male, but not female, offspring of morphine-derived fathers were much more sensitive to morphine's antinociceptive effects than controls. Morphine-derived offspring had more pronounced analgesic responses to morphine at the time of the "peak analgesic effect" and a longer duration of antinociceptive activity. These results probably cannot be explained on the basis of altered biodisposition of morphine, because the blood levels attained after morphine administration were equivalent in morphine- and saline-derived offspring. Of course, it is possible that blood levels may not accurately reflect the bioavailability of morphine at its active sites in the brain, but it is difficult to imagine what mechanism could be involved in such an effect. Rather, based upon the predominance of evidence at this point, the most parsimonious interpretation of our data is that those neuronal systems mediating analgesia, at least as assessed by the hot-plate test, were in some manner altered in male, but not female, offspring sired by morphine-treated fathers to produce an enhanced sensitivity to morphine.

The mechanisms underlying the sex differences observed in the offspring derived from morphine-exposed fathers are not understood. It is, of course, well established that female rats differ from males in their sensitivity to morphine in most analgesic assays (for reviews see Cicero *et al.*, 1989; Cicero and Adams, 1995), but it is not apparent what contributes to these sex differences. Moreover, how paternal opiate exposure might interfere with these sex-linked neurobiological differences also is unclear. However, it should be noted in a large number of earlier studies that gender-specific behavioral, endocrinological and other physiological effects also have been observed in offspring derived from alcohol- and opiate-exposed fathers (Friedler, 1975, 1985; Cicero *et al.*, 1989, 1991b; Abel, 1992, 1993; Cicero, 1994). Thus, although we can currently provide no explanation of these sex-linked differences in the effects of paternal opiate exposure, they nevertheless appear to be striking and reproducible with both acute and chronic paternal morphine administration.

Our results indicate that neither cocaine at a relatively high dose (40 mg/kg in two 20-mg/kg doses), or 1 hr of restraint stress produced any effects on fetal outcome, in terms of offspring viability or on their subsequent response to the antinociceptive properties of morphine. Because morphine, cocaine and, of course, restraint stress produced marked increases in the activity of the HPA axis, it appears unlikely that the major confound of stress or, alternatively an activation of the HPA axis which is characteristic of virtually all substances of abuse (e.g., Cicero, 1987; Cicero and Adams, 1995), can be a prominent factor in the results we observed with morphine. Whether this effect of morphine is selective to opiates, as opposed to other substances with abuse potential, is unknown at this time, but recent studies from our laboratory (Cicero, 1994; Cicero and Adams, 1995) suggest that acute paternal alcohol administration produces adverse effects on fetal outcome measures similar to those observed

in the present studies with morphine (smaller litter sizes and higher postnatal mortality rates). However, in contrast to the present results with morphine, our earlier data suggested that alcohol also influenced fertility parameters in that we observed markedly fewer pregnancies in females mated with alcohol-exposed male rats than in controls, despite normal mating behavior. These data thus suggest that paternal alcohol administration alters certain fetal outcome measures in a manner similar to paternal morphine administration but, in contrast to morphine, also produces adverse effects on fertility parameters. It remains to be determined whether other substances with significant abuse potential will produce effects similar or dissimilar to those observed with alcohol and morphine, but our results suggest that paternal drug administration can at least in some cases represent a significant risk factor in the development of their offspring.

The mechanisms underlying the effects of paternal opiate exposure on their offspring are not easily explained. However, several possibilities exist: first, it is possible that morphine acts directly as a mutagen on sperm in some as yet undetermined way; second, some "selection" of sperm may take place such that only a specific population is functionally intact after exposure to relatively high levels of morphine; or, finally, some alteration in factors secreted into semen from the seminal vesicles or prostate could influence the activity of ejaculated sperm. We are not aware of any studies in which any of the possibilities has been examined for morphine, particularly after an acute injection. However, several recent studies have shown that drugs of abuse may induce subtle mutations in sperm (Badr and Badr, 1975; Cohen, 1986; Narod *et al.*, 1988; Abel, 1992; Pylkänen and Salonen, 1992) and, in addition, it has been demonstrated that drugs and toxicants accumulate in semen and some may bind to receptors on sperm (Borski *et al.*, 1954; Gerber and Lynn, 1976; Leger *et al.*, 1977; Yazigi *et al.*, 1991; Yazigi and Polakoski, 1992). These observations suggest that abused substances may either directly impair sperm, and thereby influence the development of offspring, or be transported to the ovum *via* the seminal fluid or by directly binding to sperm where they may adversely affect the development of the conceptus. Alternatively, the biochemical and nutritional composition of the seminal fluid, which is necessary for the survival of sperm and ensuring successful conception, could also be changed by a period of morphine exposure. If either of the latter suggestions is true, then it can be postulated that the conceptus may be exposed to high levels of a potential toxicant or, alternatively, that seminal factors facilitating and maintaining the conceptus are altered during the earliest stages of development, which would as a result affect normal maturation.

At present we are unable to support or refute any of these speculative hypotheses. However, it should be noted that any mutagenic effect of morphine must have been very minor, because we found in both our earlier work (Cicero *et al.*, 1991a) and the current studies that normal pregnancy rates occurred and highly selective deficiencies in the offspring were found, indicating that what we have observed is not a dominant-lethal mutation or gross chromosomal aberration. Thus, although we certainly cannot rule out that morphine exerts toxic effects on sperm, any abnormalities are probably quite subtle and therefore will be difficult to detect.

In conclusion, we have observed that acute morphine ex-

posure in male rats has direct effects on their ability to produce normal progeny. Whether these data have any clinical relevance remains to be determined, but in view of the high prevalence of drug abuse in males of child-rearing age (Johnston *et al.*, 1987), recent evidence that the sons of drug-abusing fathers have significant endocrine, cognitive and physiological deficits compared with controls (Begleiter and Projesz, 1988; Ehlers *et al.*, 1989; Pickens *et al.*, 1991), and animal studies in our laboratory which suggest that paternal alcohol administration also produces fetal abnormalities, it seems clear that the possibility of effects of paternal morphine administration on fetal outcome should be examined more closely.

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