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Chronic Methadone Administration to Male Rats: Tolerance to Adverse Effects on Sires and Their Progeny

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SOYKA, L. F., J. M. JOFFE, J. M. PETERSON AND S. M. SMITH. *Chronic methadone administration to male rats: Tolerance to adverse effects on sires and their progeny.* PHARMAC. BIOCHEM. BEHAV. 9(4) 405–409, 1978.—Previous studies have shown that acute administration of methadone to male rats prior to mating results in adverse effects on their progeny, particularly decreased birth weights and increased neonatal mortality. Rather than chronic administration accentuating these effects, results of the present study indicate that tolerance developed so that no adverse effects were found in offspring sired after 21–32 days of methadone administration. In the sire, maintenance of normal weights of accessory sex glands after 4 months of daily methadone suggests that tolerance developed to the CNS effect(s) responsible for the depressed serum LH and testosterone levels found after acute administration of narcotics. In contrast, tolerance did not develop to the inhibition of weight gain produced by methadone administration. No evidence for a dominant lethal effect could be found after chronic methadone administration, in contrast to suggestive evidence for this effect found in previous experiments after acute methadone administration.

Methadone Narcotics Tolerance Perinatal outcome Dominant lethal Open-field test Birth weight Neonatal mortality

STUDIES from this laboratory [7, 8, 10] have established that administration of dl-methadone HCl to male rats for 2–12 days prior to their mating with drug naive females results in an increased frequency of abnormally small litters, decreased birth weights and a marked increase in neonatal mortality. When methadone was injected once per day for twelve days and the outcome of nightly matings analyzed in successive 4 day blocks, progeny of matings occurring after 1–4 days of methadone were most affected in terms of birth weight and neonatal mortality, while those of matings occurring after 9–12 days had neonatal death rates no different from offspring of control male matings [10]. The sires had lost a mean of 18 g of body weight after the 12 days of treatment, and weights of their seminal vesicles and prostates were decreased.

A possible mechanism to explain these results arises from the finding by Cicero *et al.* [2–4] that serum LH and testosterone levels were rapidly depressed by acute subcutaneous administration of morphine, methadone, and other narcotics. Testosterone levels returned to normal after 21 days of morphine administration, suggesting that tolerance had developed [4]. The present study was designed to evaluate the development of tolerance to effects on paternal weight gain and pregnancy outcome resulting from matings following chronic administration of methadone.

METHOD

Animals

Charles River CD albino rats were caged in a temperature controlled room (21 ± 1°C) artificially illuminated from 0700-1900 hr, and were allowed Purina Lab Chow and water ad lib.

Experimental Protocol

Each treatment group consisted of 4 males, 65–70 days of age at the start of the experiment. The low dose group received incremental SC doses of dl-methadone HCl in sterile water at 1000 hr of 2.5, 5, 7.5 mg/kg in 4 day blocks and finally 10 mg/kg until 136 days after the start of the experiment. The high dose group received 5, 10, 15 and then 20 mg/kg on the same schedule. The control group received equivalent volumes of sterile water SC daily. Rats were weighed daily.

Animals were killed at the end of the experiment and organs weighed.

During the first mating period, beginning at 21 days of methadone administration, males were caged with females overnight, and vaginal smears were examined the following morning for the presence of sperm. The design was to obtain 2 litters sired by each treated male and 4 litters from

each control male so that each control litter could be yoked with a methadone-sired litter. Due to the failure of some females with positive vaginal smears to deliver, 6 pregnant females were obtained from each methadone treatment group, and were matched with 2 comparable groups of 6 litters sired by control males. Since the neonatal mortality was very low in both methadone-sired groups and similar to the control groups, data from the latter were combined for presentation in the Results section.

At birth, pups were weighed, sexed, and counted. Thereafter, they were counted every 2 days until weaning at 21 days of age. Weighings were repeated at 4, 8, 16 and 21 days of age.

Dominant Lethal Test

During the second mating period, beginning after 92 days of methadone administration, each male was caged with 3 females each night for 5 nights per week for 6 successive weeks. Each morning vaginal smears were obtained and those whose smears contained sperm were caged separately to await delivery. Methadone administration was continued to avoid withdrawal symptoms during the mating period. Females with positive vaginal smears were killed at 13 days of gestation, since a pilot study of females killed from Days 11–14 showed that deciduomata were clearly distinguishable at this time. The ovaries were excised, placed in 10% Formalin and coded to allow for blind recording of corpora lutea counts. The uterine horns were inspected for deciduomata, late fetal deaths, and living implants according to the protocol recommended for dominant lethal testing of male mice by Epstein and Röhrborn [5].

Behavioral Testing

Open field testing of offspring was performed at 75 days of age as previously described [9].

Statistical Analyses

Data pertaining to sires were analyzed as one-way analyses of variance with 3 treatment levels and, except as otherwise indicated, data on offspring were analyzed as treatment by sex factorial analyses of variance. Specific a posteriori comparisons utilized the Newman-Keuls test.

RESULTS

Weight Gain of Sires

Tolerance to the inhibition of weight gain by the sires did not develop during the 4 months of daily methadone injections (Fig. 1). Weight gain of the high-dose group rapidly fell behind, so that by 8 days of treatment, their mean body weight diverged from that of the low-dose group which initially tracked the rate of gain of the control group. Though there was an upswing in weight gain of both treatment groups, which paralleled that of the control groups from Days 60 to 92, the rate of gain then slowed, possibly because of age and/or because of exposure to females. Decreased weight gain or actual loss of weight was noted in all groups during the periods of mating.

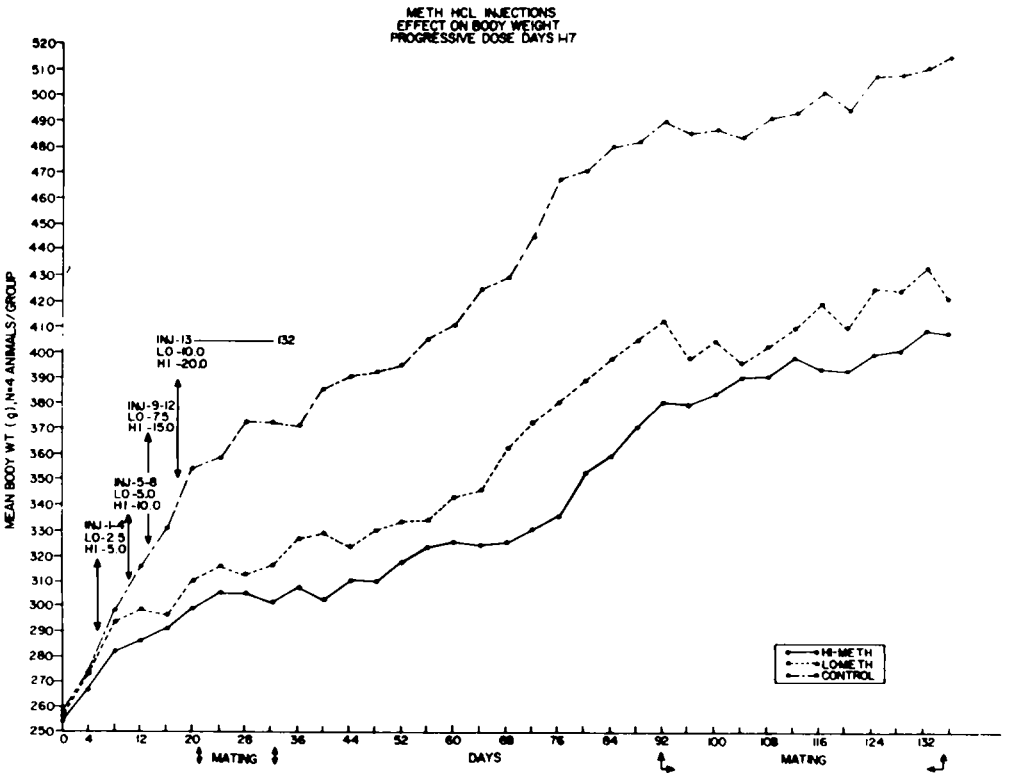

FIG. 1. Body weight of male rats during four months of daily injections of methadone. The first period of matings (indicated by the arrows) was used to evaluate effects on offspring. The second mating period consisted of 6 successive weeks of matings for the dominant lethal test. Doses were increased according to the schedule indicated on the figure.

TABLE 1

ORGAN WEIGHTS* RELATIVE TO BODY WEIGHT

	Methadone			
	Low dose	High dose	Control	p
Body weight (g)	416.8 ± 25.3	397.5 ± 13.7	498.5 ± 22.2	<0.05
Seminal vesicles	0.26 ± 0.01	0.02 ± 0.01	0.23 ± 0.02	NS
Ventral prostrate	0.17 ± 0.02	0.13 ± 0.01	0.15 ± 0.01	NS
Testes	0.77 ± 0.02	0.88 ± 0.02	0.68 ± 0.05	<0.05
Adrenals	0.014 ± 0.001	0.014 ± 0.001	0.008 ± 0.0002	<0.001

*Organ weights are expressed as a percent of body weight, mean ± S.E., N=4.

TABLE 2

OUTCOME OF PREGNANCY AFTER CHRONIC ADMINISTRATION OF METHADONE TO THE SIRE

	Methodone			
	Low dose	High dose	Control	p*
Number of litters	6	6	12	—
Litter size	14.5 ± 0.1	11.5 ± 1.3	13.3 ± 0.5	NS
Sex ratio: M/F	1.42	0.97	0.90	NS
Birth weight (g)	6.4 ± 0.1	6.7 ± 0.1	6.4 ± 0.1	<0.001
Neonatal mortality	4/81 (4.9%)	1/68 (1.5%)	2/147 (1.4%)	NS†
Weight at weaning (g)	43.2 ± 0.7	44.2 ± 0.5	44.8 ± 1.1	NS

*Significance of F value for treatment.
†From Fisher exact probability test.

Body and Organ Weights

At autopsy, the body weights of the methadone treated sires were significantly lower than those of controls, $F(2,9)=6.55$, $p<0.05$ (Table 1). Although the seminal vesicles and ventral prostates of males in the high dose group weighed less, when expressed relative to body weight they did not differ from controls (Table 1). The weight of the testes did not differ among the groups, but the testes of the high dose group were significantly heavier than were those of controls when expressed relative to body weight, $F(2,9)=9.28$, $p<0.05$. The adrenal glands of both methadone groups were markedly enlarged, $F(2,8)=15.08$, $p<0.001$.

Pregnancy Outcome

Litters sired after chronic methadone administration were of normal average number and sex ratio (Table 2). Progeny of the high-dose males were heavier at birth than those sired by controls or males of the low-dose group, $F(2,310)=8.04$, $p<0.001$, in contrast to the decreased birth weights relative to controls found after acute methadone dosing regimens [7,10]. Progeny of low-dose males did not differ significantly from controls upon a posteriori testing. Male pups were significantly heavier than females, $F(1,310)=23.82$, $p<0.01$. Neonatal mortality was very low in all 3 groups, ranging from 1–5%. Males were heavier than females at weaning, $F(2,283)=6.28$, $p<0.01$, but no intergroup differences were found.

Behavioral Studies

Activity scores in the open-field showed a difference between sexes, $F(1,183)=29.55$, $p<0.001$, but no significant treatment effect (Table 3). Defecation scores differed by sex, $F(1,138)=31.46$, $p<0.001$, and by treatment, $F(2,138)=11.46$, $p<0.001$, with the highest scores for both sexes found in the progeny of the low-dose group. Among high-dose progeny only the males had scores significantly higher than controls. The treatment effect on defecation was specific to the open-field test since the home-cage defecation scores did not differ as a result of drug treatment, though the difference between the sexes persisted, $F(1,42)=10.56$, $p<0.01$.

Dominant Lethal Test

Females mated with control males produced the greatest number of deciduomata, the primary indicator of dominant lethal mutations. Though the number of females impregnated each week varied within and between groups, the totals were equivalent (Table 4). No trend over the six-month period was seen in any of the parameters. The mean number of early fetal deaths (deciduomata/rat) was: low-dose, 0.65; high-dose, 0.89; and control, 1.16, or relative to the corpora lutea count, 4.5, 6.2 and 7.5%, respectively. Analysis of covariance on the number of viable eggs and the apparent litter sizes of the control versus the treatment groups did not reveal statistically significant differences.

DISCUSSION

The development of tolerance to some, but not all, pharmacologic effects is characteristic during the chronic administration of narcotic analgesics and their synthetic analogues,

TABLE 3

OPEN FIELD TESTING OF PROGENY OF METHADONE TREATED MALE RATS

Open-field	Methadone Low dose	Methadone High dose	Control	p*
Activity†	223 ± 9.7	234 ± 10.8	231 ± 8.6	NS
Male	204 ± 13 (18)	199 ± 13 (18)	199 ± 10 (36)	
Female	243 ± 13 (18)	269 ± 13 (18)	264 ± 12 (36)	
Defecation score‡	10.9 ± 0.8	7.2 ± 0.9	6.0 ± 0.6	< 0.001
Male	13.3 ± 0.1	10.8 ± 1.1	7.3 ± 1.0	
Female	8.4 ± 1.2	3.6 ± 1.0	4.8 ± 1.0	
Home cage defecation score§	216 ± 7.7	217 ± 8.5	199 ± 5.4	NS
Male	232 ± 10 (6)	229 ± 13 (6)	209 ± 7 (12)	
Female	200 ± 8 (6)	205 ± 9 (6)	190 ± 7 (12)	

*Significance of F value for treatment.
†Number of blocks in grid crossed in 4 × 2 min trials, mean ± S.E. (N).
‡Boli/4 × 2 min trials.
§Boli/2 × 24 hr; 3 rats/cage.

TABLE 4

DOMINANT LETHAL TEST AFTER CHRONIC METHADONE

Week	N females	Ova failed to implant†	Early fetal deaths*	Late fetal deaths	Living implants	Corpora lutea count	Viable ovulations (%)
LO Dose Methadone Sired							
1	8	13	8	2	87	110	79.1
2	9	4	3	—	125	132	94.7
3	7	19	5	—	78	102	76.5
4	12	20	12	—	140	172	81.4
5	7	2	1	—	103	106	97.2
6	9	15	5	—	108	128	84.4
Totals	52	73 (9.7%)	34 (4.5%)	2	641	750–14.4/rat	85.5
HI Dose Methadone Sired							
1	6	8	8	—	71	87	81.6
2	9	3	6	1	119	129	92.2
3	12	21	4	3	152	180	84.4
4	9	5	7	—	120	132	90.9
5	10	16	10	2	116	144	80.6
6	10	11	15	1	107	134	79.9
Totals	56	64 (7.9%)	50 (6.2%)	7	685	806–14.4/rat	85.0
Control Sired							
1	8	18	11	2	86	117	73.5
2	9	7	13	—	116	136	85.3
3	12	19	8	1	156	184	84.8
4	8	4	16	—	103	123	83.7
5	10	26	12	1	118	157	75.2
6	11	21	7	—	139	167	83.2
Totals	58	95 (10.7%)	67 (7.5%)	4	718	884–15.2/rat	81.2

†Ova failed to implant is the difference between the corpora lutea count and all other measures.
*Early fetal deaths are the deciduomata count.

such as methadone. In volunteer post-addicts, at least partial tolerance develops to the anorectic, sedative and respiratory depressant effects of oral or SC methadone, but less fully to the miotic and constipative effects [6]. The precise basis for this phenomenon remains obscure.

From our results it appears that tolerance did not develop to the growth inhibiting action of methadone in the rat. While visibly sedated for a few hours post-injection during the first few days of methadone administration, the rats rapidly developed tolerance to this effect and subsequently their post-injection motor activity and behavior appeared to be grossly normal. Decreased weight gain occurred acutely, at least in the high-dose group, and continued during the 4 months, despite maintenance on a relatively low fixed-dosage regimen.

The acute response we noted contrasts with the maintenance of normal body weight and food and water intake reported by Cicero and coworkers [2] for 3 days following SC implantation of a 75 mg morphine pellet. Since in that study plasma testosterone fell sharply, the weight gain inhibiting effect appears to be separable from the neuroendocrine effect, presumably exhibited at the hypothalamic-pituitary level.

Our finding of normal weights for prostate and seminal vesicles relative to body weight strongly suggests the presence of adequate testosterone secretion at the time of sacrifice, consistent with the tolerance to the testosterone-lowering effect reported after 20 days of morphine administration to rats [3]. Tolerance to the testosterone-lowering effect apparently also develops in man, since many men maintained on methadone have normal serum testosterone and LH levels and are fertile [6]. Moreover, the testes weights of the methadone treated rats did not differ from controls, a finding compatible with adequate gonadotropin secretion.

The usual acute effect of opioids on ACTH release is depression. Patients treated chronically with morphine have decreased plasma and urinary 17-hydroxycorticosteroid concentrations [6]. However, the adrenals of our rats were markedly enlarged in both groups of methadone-treated rats. If such enlargement signified increased corticosterone secretion this effect might explain the reduced growth rate observed.

Significant differences were found between treatment groups in the open-field defecation scores as found after acute administration [10]. These data indicate that the basis for this effect on progeny was therefore separable from the mechanism(s) responsible for alterations in litter size, birth weight, and neonatal survival towards which tolerance developed.

That the offspring of sires treated chronically with methadone had normal birth weights coupled with data from a previous study [10] all support the view that tolerance develops to whatever mechanism(s) underlies the depressant effect observed after acute treatment. While the offspring of the high-dose group had birth weights which exceeded those of the controls we are unwilling to ascribe this to a novel drug effect without additional documentation. Most importantly, their birth weights were not decreased. The mechanism whereby tolerance developed to the adverse effect on neonatal mortality may be related to the lack of depression of birth weight after chronic methadone. That such tolerance relates to the return of the testosteone levels to normal during chronic administration is an appealing proposal, consistent with our results and currently under investigation.

ACKNOWLEDGEMENT

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