Pharmacology Biochemistry & Behavior, Vol. 22, pp. 949–953, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Opiate Self-Administration in Adult Offspring of Methadone-Treated Female Rats

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Received 2 June 1982

HOVIOUS, J. R. AND M. A. PETERS. *Opiate self-administration in adult offspring of methadone-treated female rats.* PHARMACOL BIOCHEM BEHAV 22(6) 949–953, 1985.—Chronic treatment of female Sprague-Dawley rats with methadone 5 mg/kg IP throughout gestation and lactation resulted in an increased oral self-administration (S.A.) of morphine by their 85–90-day-old offspring. By day 16 of the S.A. treatment schedule methadone offspring were taking 75 to 80% of their total fluid intake as morphine solution when given a choice between morphine solution and water, while control offspring under the same conditions took 33% of their total fluids as morphine solution. When the subjects were again given a choice between water and morphine solution following a 12-day drug free period, methadone offspring drank a significantly greater percentage of morphine solution than controls. Methadone S.A. in methadone offspring was not different from controls. The reasons for this marked difference between morphine and methadone S.A. are not clear. However, it does appear that chronic maternal exposure to methadone may facilitate development of a morphine-S.A. behavior in their offspring.

Methadone Morphine Maternal opiate exposure Offspring self-administration Perinatal biochemistry

PREVIOUS studies have reported that treatment of female rats with opiates such as methadone or morphine during intrauterine and early postnatal development results in behavioral [9, 13, 18, 19], physiological [12,16], and biochemical [8, 11, 13] changes in the offspring. Treatments caused decreases in body and brain weight, as well as decreases in brain DNA, RNA and protein content [10,13]. These biochemical changes in the offspring can be correlated with treatment-related changes in thermoregulation [16], decreased ability to learn and/or perform in shock-avoidance and discrimination learning tasks [9,13], with an altered analgesic response to morphine [13] and methadone [19], facilitation of morphine self-administration in offspring of morphine-treated dams [2], and alterations in the levels or binding capability of opiate receptors [3, 4, 17]. Although cause-effect relationships have not been established, the changes observed in brain chemistry may be related to and underlie the alterations seen in the physiological and behavioral responses of these offspring.

Clinical investigators have observed that parental behavior as it relates to child care, drug use, marriage compatibility, etc. significantly affects the way their children behave when exposed to the opportunity to experiment with drugs [6,15]. From these observations it has been suggested that the primary factors responsible for offspring use of narcotics are the home, drug availability, psychological health, and socioeconomic environment [6]. While these factors are no doubt significant, the biochemical and behavioral studies referred to earlier along with the study presented here, would suggest that a biochemical predisposition may exist independent of the environment, and that this predisposition may be detected using animal models, where offspring of drug-treated mothers are forced and/or allowed free access to the drugs by self-administration [1,5]. Studies using animal models, however, cannot always be interpreted directly in terms of clinical relevance. For instance, most animals will not voluntarily initiate drug intake [1, 5, 7, 8, 14]; consequently, in order to encourage oral S.A. of drugs by way of their drinking water the animals need to be initially "forced" to take the drug solution. In our study the animals were forced to drink drug solution by periodically removing all fluids for a 24-hour period prior to providing only drug containing solution to drink following the schedule outlined in Table 1. Drug perference was identified by providing time periods when a choice between drug solution and water was available for S.A.

The specific questions addressed in this study were: (1) Do offspring of methadone-treated dams show an increased tendency or desire to drink drug solution when given a choice between water and drug solution? (2) Is there a treatment-related difference in the development or onset of this tendency? (3) Does a preference for drug solution continue to exist following a drug-free period?

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TABLE 1

SCHEDULE OF TRIALS FOR ANIMALS GIVEN A CHOICE*

Trial (T)	W	W	N	F	C	F	C	F	C	N	F	F	C	F	F	C
Day on Treatment (D)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Trial (T)	N	F	F	C	C	F	N	F	F	C	F	F	F	C	W	C
Day on Treatment (D)	17	18	19	20	21	22	23	24	25	26	27	28	29	30–34	35–45	46

*Each trial was given for a 24-hour period. T refers to the type of trial or solution(s) the animals had available. W indicates that both bottles contained water. N indicates that no fluids were available. This trial was designed to ensure fluid intake during certain portions of the pretreatment phase (F). F indicates that both bottles contained the test solution, either water (for drinking control groups), morphine or methadone. C indicates that animals on drug solution were given one bottle filled with water and the other filled with either morphine or methadone solution, the animls could choose water or drug solution whichever they preferred. This periodic assessment was designed to determine the onset and intensity of drug-seeking behavior.

METHOD

Experimental subjects used in these studies were offspring of female Sprague-Dawley rats which had received a daily IP injection of saline (controls), or methadone (5 mg/kg). Injections started one week prior to mating, and continued until the offspring were weaned at 22 days postpartum.

Twenty females weighing approximately 250 g and males weighing approximately 350 g were obtained from Simonsen Laboratories, Gilroy, CA and were housed in stainless steel wire bottom cages in a temperature-controlled room (22±1°C) having a 12-hour light-dark cycle. The animals received a commercial Purina Lab Chow diet and water ad lib. One week prior to mating the females were randomly divided into two groups and started on treatment. Animals of the one group received a daily IP injection of saline (control) while the other received methadone (5 mg/kg). Mating was accomplished by placing one male with 2 or 3 females for 5 days each week for 3 weeks. Adult females were weighed every 2 to 3 days so adjustments in dosage could be made. The rapid weight gain that starts 5 to 6 days before delivery also served to provide an approximation of delivery dates. Approximately three days prior to anticipated delivery (based on weight gains), females were transferred to individual shoebox-type cages containing pine wood shavings (obtained from Long Beach Shavings Co., Long Beach, CA) where females were allowed to deliver and care for their young. Litter size was maintained at an average of 9 pups through culling and within-treatment-group fostering when possible. No litter contained more than 10 or less than 7 pups. Pups were weaned on day 22 postpartum and were housed in wire-bottom cages with free access to food and water until needed for experimentation.

At 85 to 90 days postpartum, one male offspring was randomly selected from each of 5 control and 5 methadone litters for testing their susceptibility to S.A. of either morphine or methadone solutions. The design of our study was similar to the study reported by Nichols [6] with the exception that our animals were given drug solution for 1, 2 or 3 consecutive days prior to choice days, following the schedule as outlined in Table 1, instead of only one day.

Each test subject was placed in an individual cage containing excess food and two bottles filled with water. After an initial two-day adjustment period the water bottles were removed for a 24-hour period prior to starting the animal on the drug exposure-testing procedure. As shown in the schedule in Table 1, the animals were then subjected to a series of "forced" trials, during which both bottles contained the test solution (either morphine or methadone at 0.5 mg/ml or water), followed by "choice" trials in which one bottle had the test solution and the other contained water. The relative position of the bottles containing water vs. test solution were randomized in order to prevent choice of solution based on position of the bottles in the cage. Three sample groups of 5 animals each from the control and methadone groups were used to compare morphine S.A., methadone S.A., and water consumption. (The water group served as a control for fluid intake.) A total of 6 groups were used. Both of the bottles and the test subject were weighed daily and the drug solution intake was calculated as both ml/kg, and as percent of total daily intake. The data were analyzed using an analysis of variance (completely randomized, between-within design) with specific comparisons being made using the Student's t-test. Significance level was $p<0.05$.

RESULTS

The data presented in Fig. 1 show the intake of morphine solution by S.A. in adult offspring of control and methadone-treated rats on days when the offspring were given a choice between water and morphine solution (0.5 mg/ml). During the first 13 days on the schedule there were no differences between the two treatment groups. A marked increase in morphine S.A. occurred in the offspring of methadone-treated dams on schedule day 16 which remained throughout the remainder of the testing procedure.

Expressing the drug solution intake as a percent (%) of the total fluid intake on choice days we obtained curves which resembled those seen in Fig. 1. Both control and methadone offspring took 20% or less of their total fluid intake as morphine solution on schedule day 9 while by day 16 methadone offspring increased their intake to 76% of their fluid intake with an average of 65±4% for days 16 to 34. Control offspring took approximately 25% on day 16 and averaged 33±3% for days 16 to 34.

Figure 2 compares the S.A. of morphine (0.5 mg/ml) and methadone (0.5 mg/l) solutions in offspring of control and methadone-treated dams. Morphine S.A. was significantly greater than methadone S.A. in both maternal treatment

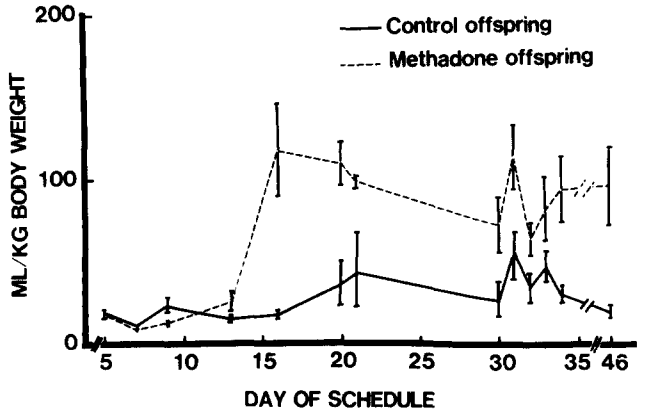

FIG. 1. Morphine self-administration (S.A.) on days when animals could choose between water and morphine (0.5 mg/ml) solution. The unbroken line represents offspring of female rats which received daily injections of saline while the broken line represents offspring of female rats which received daily injections of methadone 5 mg/kg. The data points are the mean±S.E. for 5 animals. The two lines are significantly different at $p=0.002$.

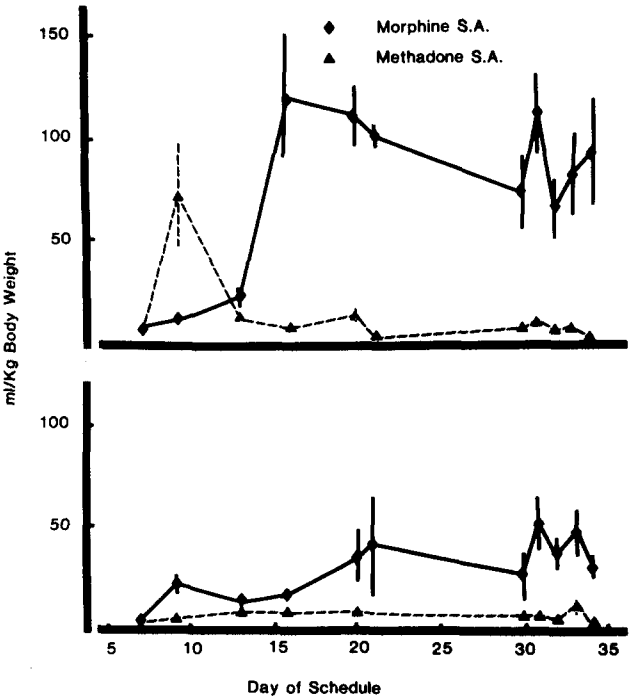

FIG. 2. Comparison of methadone vs. morphine S.A. on choice days in methadone offspring (top panel) and control offspring (lower panel). The data points are the mean±S.E. for 5 animals. Morphine S.A. is significantly greater than methadone S.A. in both control ($p=0.013$) and methadone ($p=0.001$) offspring.

groups (Control offspring, $p=0.013$; Methadone offspring, $p=0.001$). A comparison of methadone S.A. between maternal treatment groups showed no difference.

The total daily fluid intake fluctuated from day to day depending on the schedule and the drug solution available. No significant treatment-related differences in fluid intake were observed (Table 2). However, animals in the methadone S.A. groups tended to drink less total fluids on non-choice days than on choice days, indicating some reluctance to drinking the methadone solution, while animals in the morphine S.A. groups tended to drink more fluids on non-choice days than on choice days. Methadone offspring on either methadone S.A. or morphine S.A. groups tended to drink more total fluids than their corresponding control groups on both choice and non-choice days. All animals lost weight during the initial two weeks and then gained similar amounts prior to termination of the experiment (Table 3). No significant treatment-related differences in weight loss or gain were observed. This would suggest that nutritional or hydration status of the animals in the different treatment groups were not sufficiently different to account for the differences in observed responses.

TABLE 2

AVERAGE FLUID INTAKE

	Self-Administration Groups* (Test Solution)		
	Water	Methadone	Morphine
Control offspring group	A	B	C
Choice Days	127 ± 9	123 ± 6	107 ± 8
Non-Choice Days	128 ± 8	98 ± 13	125 ± 17
Methadone offspring group	D	E	F
Choice Days	127 ± 8	131 ± 11	136 ± 10
Non-Choice Days	128 ± 8	117 ± 16	140 ± 19

*Each value represents the average total daily fluid intake ± S.E. for 5 different animals and is expressed as ml/kg of body weight. Fluid consumption on "non-choice" days average the fluid consumed on "forced" days with days when no fluids were available (see Schedule, Table 1). On choice days the animals were given free access to both water and test solution while on forced days only drug solution was available. Groups A and D received water only; groups B and E received water and/or methadone; groups C and F received water and/or morphine.

DISCUSSION

Reports from our laboratory [9,10] and the laboratories of others [13,19] have demonstrated that chronic maternal exposure to methadone and morphine causes a decrease in brain protein, RNA and DNA in the offspring. These chemical changes are correlated with a decreased shock-avoidance learning and with alterations in other behavioral tasks which tend to persist into early adulthood.

A report by Glick *et al.* [2] indicated that *in utero* exposure to morphine has a significant effect on the development of intravenous self-administration behavior. These investigators concluded that, although sensitivity to morphine was not altered, *in utero* exposure facilitated the rate at which morphine self-administration behavior was learned. Our study presented in this manuscript was designed to evaluate the effects of chronic maternal methadone exposure on opiate-seeking behavior. We found that offspring of females chronically exposed to methadone during gestation are more susceptible, or predisposed, to develop a morphine-seeking behavior than are offspring of non-exposed dams (Fig. 1). This increased susceptibility or predisposition is induced dur-

TABLE 3

WEIGHT OF ANIMALS IN SELF-ADMINISTRATION GROUPS*

Maternal Treatment	Morphine			Methadone		
	Initial	Lowest	Final	Initial	Lowest	Final
Control	306 ± 28	295 ± 27	350 ± 31	280 ± 18	275 ± 19	348 ± 26
Loss/gain	—	11 ± 2	44 ± 10	—	5 ± 2	68 ± 11
Methadone	295 ± 14	277 ± 20	341 ± 10	295 ± 14	277 ± 20	341 ± 10
Loss/gain	—	13 ± 1	59 ± 8	—	18 ± 7	46 ± 16

*Each value represents the mean weight or change in weight in grams ± S.E. for five animals. Each animal was weighed daily prior to changing to a new treatment. The values presented were taken prior to starting the animals on treatment (see Table 1), at the time the animal had lost the most weight, and following day 34 of the schedule. Weight gain of control and methadone offspring on water only was 76 ± 9 and 57 ± 9 g respectively.

ing fetal and/or early postnatal development and persists into early adulthood.

Interestingly the choice of opiate used as the drug testing for susceptibility to opiate-seeking behavior can give markedly differing results (Fig. 2). Although maternal methadone treatment appeared to enhance morphine S.A. in the offspring of treated females, such treatment did not enhance methadone S.A. The reasons for differences in methadone S.A. and the S.A. of morphine is not clear at the present time; however, in searching for possible explanations for our observations, we have found that induction of oral methadone S.A. using techniques similar to those described has not been reported while morphine S.A. can be readily induced by this means [17]. These differences may be a reflection of the differences between the two drugs in taste, reinforcement characteristics, potency, disposition, pharmacokinetics, and/or to a difference in the development of the various specific opiate receptors [17] or endogenous opioids [11].

The fluid intake on choice days in both methadone S.A. groups (Fig. 2) consisted of more than 90% water during the final 24 days of the treatment schedule with 96 to 100% of the total fluid intake being water on the last day. Both control and treated animals tended to avoid drinking the methadone solution when possible (choice days) and tended to drink less fluids when only methadone solution was provided than they would otherwise drink if water was available.

The weights of the animals in the different groups fluctuated from day to day, however, overall weight gain or loss was not different from one group to the next providing little if any explanation for the differences in morphine vs. methadone S.A.

Preliminary data from our laboratory suggest that the level of β-endorphin immunoreactive substances in the pituitary and other brain regions in offspring of methadone-treated dams may be reduced. In addition there is a decrease in methadone binding to certain brain tissues. These observations, in addition to those presented in this manuscript, would suggest that chronic intrauterine exposure to methadone may result in quantitative and/or qualitative changes in the opioid systems in the offspring which modifies their behavior.

ACKNOWLEDGEMENT

The authors wish to express appreciation to Eli Lilly Company for providing the methadone used in this study.

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