Pharmacology Biochemistry & Behavior, Vol. 21, pp. 555–559, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Analgesic Effect of Opiates in Offspring of Opiate-Treated Female Rats

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Received 25 June 1982

HOVIOUS, J. R. AND M. A. PETERS. *Analgesic effect of opiates in offspring of opiate-treated female rats.* PHARMACOL BIOCHEM BEHAV 21(4) 555–559, 1984.—The purpose of this study was to determine the effect that chronic maternal exposure to narcotics has on the effectiveness of analgesic drugs in the offspring. No exposure related differences were observed in the base level response to the noxious stimuli used. However, offspring of methadone and morphine-treated mothers tended to show decreased response latencies compared to control offspring, in both the hotplate and tail flick test, following the subcutaneous administration of narcotic analgesic drugs. In all groups studied, methadone offspring had significantly reduced latencies while morphine offspring had latencies that were intermediate between the control and methadone-treated groups (in general, control latencies > morphine latencies > methadone latencies). Morphine offspring were significantly different from the controls only in the 120-day-old female group. Treatment-related decreases in the effectiveness of the analgesics in both 25- and 120-day-old offspring suggest that exposure of developing rat pups to narcotics during gestation and early postnatal development is associated with long-lasting alterations in those processes involved with pain perception and/or interpretation.

Maternal opiate dependence Morphine Offspring analgesia Methadone

CHRONIC exposure of women and suitable animal models to narcotic drugs during gestation and lactation is associated with significant behavioral changes in the offspring. Some of these changes disappear within days following delivery, while others persist for years or into young adulthood [4]. The behavioral changes [8, 9, 11, 12, 16] observed in animal models chronically treated with narcotics have been accompanied by significant decreases in brain weight, DNA, RNA and protein [3, 6, 8], suggesting to us a possible cause-effect relationship.

Recent studies in our laboratory [10] have shown that offspring of methadone-treated females will self-administer 75 to 80% of their total fluid intake as morphine solution at a time when control off-spring, on an identical self-administration schedule, self-administer 20 to 25% of their fluid intake as morphine solution. These observations provide additional evidence supporting the hypothesis that the biochemical deficits seen may be associated with a relatively selective alteration in the morphine-receptor interaction. An alteration in the pain-response following an analgesic dose of narcotics would provide another piece of evidence to help establish the validity of this hypothesis.

Several reports have shown offspring of morphine-treated females to be relatively tolerant to the analgesic effects of morphine [3, 5, 6] while other reports studying offspring of methadone-treated females have found these offspring to be more sensitive to the analgesic used [13, 14, 15]. These observations would suggest that maternal methadone and morphine (although both drugs cause a decrease in brain protein, RNA and DNA) affect the developing brain receptor response mechanisms in different ways resulting in hyperalgesia and hypoalgesia respectively.

This apparent difference is indeed intriguing and invited additional study. Consequently, the study presented here was designed to determine the analgesic effectiveness of methadone and morphine in offspring of both methadone and morphine-treated females raised and tested under identical conditions.

METHOD

The experimental animals used in these studies were mature Sprague-Dawley rats and their offspring. Virgin females weighing approximately 250 g and males weighing about 350 g were obtained from Simonsen Laboratories (Gilroy, CA) and were housed in wire-bottom cages in an air conditioned room (20–24°C) on a 12-hour light-dark cycle. The animals received a commercial Purina Lab Chow diet and water ad lib.

After a 2-day acclimatization period the females were divided into three treatment groups. Group 1 received an IP injection of methadone (5 mg/kg); Group 2 received an IP injection of morphine (5 mg/kg); and Group 3 received an equal volume of saline (the vehicle used to dissolve the drugs) by the same route. Treatment of the females continued until the offspring were weaned at 21 days post-partum. The reasons for using the IP route of administration and the precautions used have been described earlier [7].

One week after beginning treatment, one male rat was placed with two females for breeding. The female rats were

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TABLE 1

RESPONSIVENESS OF OFFSPRING TO THE TESTING PROCEDURE*

Maternal Treatment	Response Latency					
	Hot Plate			Tail Flick		
	Total	Male	Female	Total	Male	Female
Control (Saline)						
25 days	5.92 ± 0.27	5.59 ± 0.31	6.81 ± 0.42	7.21 ± 0.27*	7.59 ± 0.42†	6.99 ± 0.35
120 days	5.42 ± 0.19	5.27 ± 0.29	5.55 ± 0.25	5.47 ± 0.22	5.76 ± 0.31	6.99 ± 0.30
Methadone						
25 days	5.89 ± 0.17	5.92 ± 0.26	5.86 ± 0.24	7.02 ± 0.21†	7.23 ± 0.31	6.83 ± 0.28†
120 days	5.44 ± 0.20	5.53 ± 0.29	5.36 ± 0.28	5.68 ± 0.29	6.10 ± 0.49	5.26 ± 0.28
Morphine						
25 days	5.68 ± 0.15	5.90 ± 0.21	5.36 ± 0.20	7.58 ± 0.25†	7.65 ± 0.42†	7.12 ± 0.40†
120 days	5.45 ± 0.16	5.31 ± 0.24	5.53 ± 0.27	5.25 ± 0.22	5.44 ± 0.38	5.07 ± 0.24

*All offspring were tested in both tests prior to administration of the analgesic and the time required for the animals to show signs of discomfort was recorded. Values are the means ± SE in seconds.

†Significantly different from 12-day-old animals.

weighed at the time of mating and every 3 to 4 days thereafter. When the female had gained 100 g she was placed in a 9×9×18″ clear plastic shoe-box type cage containing pinewood shavings (obtained from Long Beach Shavings Co., Long Beach, CA), suitable for delivery and caring for her offspring. Litter size was maintained at 7 to 10 pups through culling and within-treatment fostering. The offspring were weaned when 21 days old and placed in a wire-bottom half-stock cage until used in the experiment. Male and female offspring were housed separately with 6 animals per cage.

Animals from each group were tested for analgesia to methadone and morphine using the hotplate [1] and tail flick [2] testing procedures at age 25 days and again at age 120 days. The number of animals used in each group varied but was approximately 16 in the 25-day-old groups and 6 in the 120-day-old groups. Analgesic response of male and female offspring were analyzed together in the 25-day-old groups but were analyzed separately by sex in the 120-day-old groups.

The hotplate testing procedure consisted of placing the animal on the flat surface of a metal container filled with boiling acetone at 55°C. The endpoint was reached when the animal showed signs of distress or anxiety such as licking of paws, jumping, rapid withdrawal of paws, or after a time lapse of 60 seconds. Following the hotplate test, the subject was placed in a restraining cage and its tail was blackened and suspended approximately one-half inch above a 200 watt projector bulb as the heat source. The heat source was removed following tail withdrawal or after 20 seconds without response. Latency of response in both the hotplate and tail flick tests was determined using a hand-held stopwatch and was recorded to the nearest 0.1 second with a maximum time of 60, or 20 seconds for the hotplate or tail flick test respectively.

After initial testing in the absence of drugs (time zero) each animal was given a suprascapular subcutaneous injection of either methadone, morphine or saline. The 25-day-old animals were given methadone or morphine 1 mg/kg while 120-day-old offspring received 2.5 mg/kg methadone or 3.5 mg/kg morphine. Each animal was tested on the hotplate at 15, 30, 60, 90 and 120 minutes following injection and at 20, 35, 65, 95 and 125 minutes with the tail flick test.

Treatment-related differences were identified using a one way Anova, with specific comparisons being made using the student's t-test. Significance level unless otherwise indicated is $p<0.05$.

RESULTS

Initial response (time zero) latencies prior to drug exposure in both the hotplate and tail flick tests showed no treatment-related differences. However, 25-day-old animals tended to have longer latencies in the tail flick testing than did the 120-day-old offspring (Table 1). This was true for both male and female offspring with no significant sex differences in initial response latencies in either the 25- or 120-day-old offspring.

Figure 1 shows the analgesic effectiveness of the doses of methadone and morphine used in the 120-day-old offspring using the tail flick test. As can be seen, 2.5 mg/kg methadone caused a greater response latency than did 3.5 mg/kg morphine. However, the dosage of both drugs was sufficient to produce a significant increase in latency of response (analgesic effect) in control offspring. Similar responses were observed for all maternal treatment groups at both 25 and 120 days of age in both the hotplate and tail flick tests.

Although the response of the various offspring to the hotplate and tail flick tests was not different at time zero, significant differences were seen when maternal treatment groups were compared for overall analgesic effectiveness (average latency over the testing period) and at the time of peak analgesic effect (maximum latency). As can be seen in Figs. 2 and 3, control offspring generally showed the longest latencies (greatest effect of the drug) while morphine offspring were intermediate and methadone offspring showed the shortest latencies.

Data presented in Fig. 2 show the average, or overall analgesic effect and the peak analgesic effect in the 25- and 120-day-old offspring from each maternal treatment group

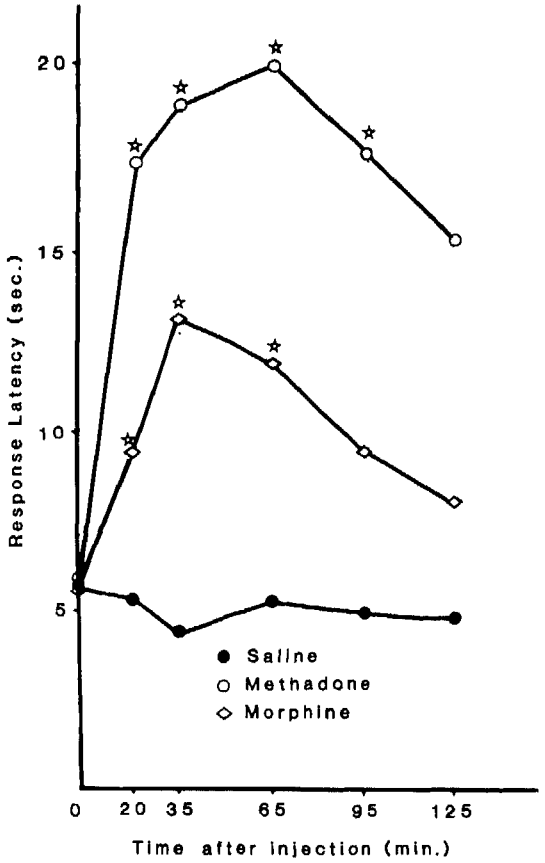

FIG. 1. Data presented show the response latency using the tail flick test in 120-day-old offspring in saline-treated dams following a subcutaneous injection of saline, morphine 3.5 mg/kg, and methadone 2.5 mg/kg. *Indicates a difference from saline injected $p<0.05$.

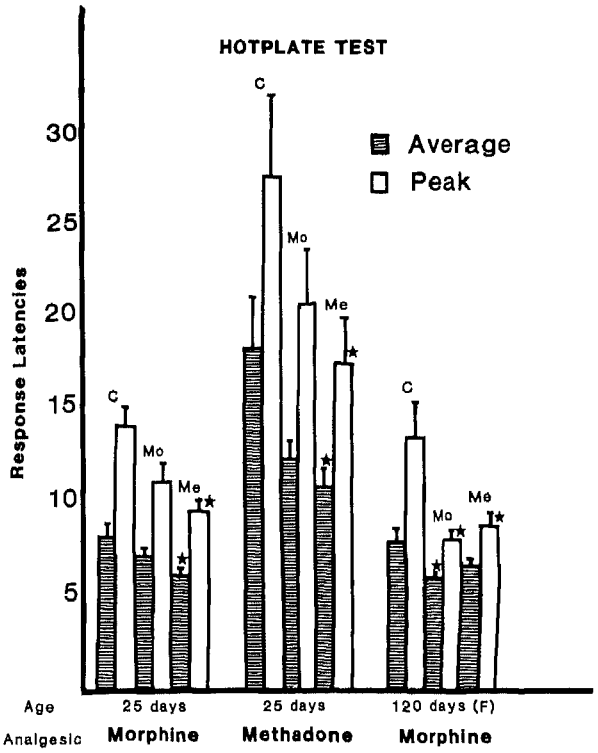

FIG. 2. Data presented show the mean±S.E. response latencies (seconds) or analgesic effect of morphine and methadone in offspring of saline (C), morphine (Mo), and methadone (Me) treated dams using the hotplate test for analgesia. Dams were started on treatment prior to mating and continued until offspring were weaned. Average (striped box) refers to the overall analgesic effect and is a combination of the peak and duration characteristics. Peak (open box) is the maximum response latency. There were 16 animals (males plus females) per treatment group in the 25-day-old animals and 6 female (F) offspring per treatment group in the 120-day-old animals. *Indicates a difference from control $p<0.05$.

using the hotplate test. Both male and female 120-day-old offspring of morphine and methadone mothers showed a decreased latency of response in both the hot plate and tail flick tests, however, the 120-day-old females response on the hot plate was unique in that the response latencies in the morphine offspring were shortest rather than being intermediate as usual. In all instances, in the hotplate testing with either morphine or methadone analgesia, the methadone offspring showed a significant decrease in response latency. Morphine offspring were significantly different from controls only in the 120-day-old female offspring tested for morphine analgesia. However in no instance, when using the hotplate testing procedure, were morphine offspring statistically different from methadone offspring in the analgesic response.

The response to analgesic drugs using the tail flick test (Fig. 3) was generally similar, with the methadone offspring being statistically different from control offspring with both morphine or methadone analgesia. Again no differences were seen between morphine offspring and controls. The only significant difference seen between morphine and methadone offspring was in the 25-day-old animals tested with methadone analgesia.

DISCUSSION

The study presented here demonstrates that chronic exposure of female rats to methadone or morphine during the perigestational period results in a significant decrease in response latency in the hotplate and tail flick tests following the administration of analgesic doses of either methadone or morphine. In general, the data show that offspring of methadone-treated dams had shorter latencies than morphine offspring which in turn had shorter latencies than control offspring (i.e., methadone < morphine < control). In all instances methadone offspring had statistically shorter latencies than controls. However, with few exceptions, the differences seen between control and morphine offspring or between morphine and methadone offspring were not significant. The two instances where morphine offspring were significantly different from control or methadone groups were seen in the 120-day-old females tested on the hotplate with morphine as the analgesic (Fig. 2), and in the 25-day-old offspring tested for methadone analgesia using the tail flick testing procedure (Fig. 3). The 25-day-old methadone offspring showed a significantly reduced response latency com-

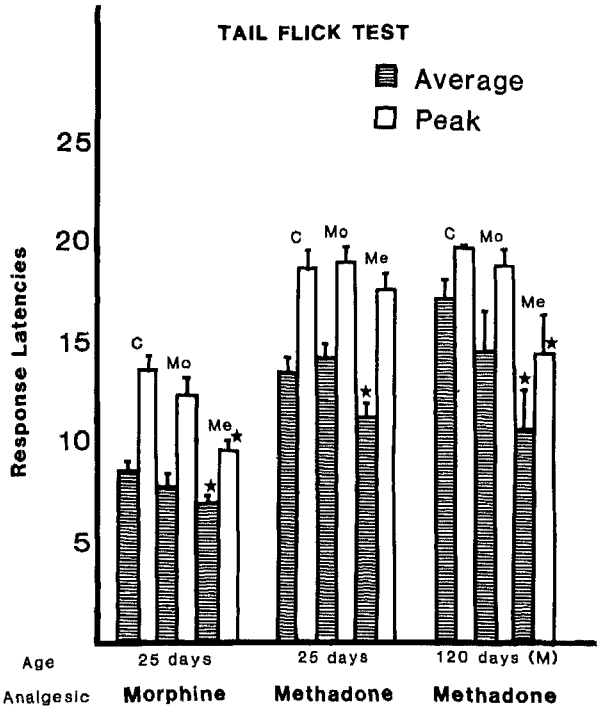

FIG. 3. Data presented demonstrate the mean±S.E. response latencies (seconds) or analgesic effect of morphine and methadone in offspring of saline (C), morphine (Mo), and methadone (Me) treated dams using the tail flick test for analgesia. Females were treated starting prior to mating and continued until offspring were weaned. Average (striped box) refers to the overall analgesic effect across time and is a combination of the peak and duration characteristics. Peak (open box) is the maximum response latency. There were 16 offspring (males and females) per treatment group in the 25-day-old animals, and 6 male (M) offspring per treatment group in the 120-day-old animals. *Indicates a difference from control.

pared to both morphine and control offspring. These changes would suggest that perinatal exposure to narcotics may result in the development of an inherent long-lasting tolerance to opioid substances in the offspring. This enhanced tolerance is consistent with previous studies with perinatal exposure to morphine offspring [3, 6, 10], but tend to disagree with studies using offspring of methadone exposed dams [5, 13, 14]. Based on the biochemical deficits [3, 6, 8] and behavioral alterations [8, 9, 11, 12, 16] observed in offspring of opiate-treated mothers, we expected to see similar effects in the two treatment groups (i.e., decreased analgesic effectiveness).

The decreased brain protein, RNA and DNA [3, 6, 8], the decreased learning ability [9], and the increased susceptibility toward self-administration of morphine [10] observed in offspring of narcotic-treated mothers combined with the decreased analgesic effectiveness of methadone observed in this study, suggest that intrauterine exposure to narcotic drugs may have a significant effect on those areas of the brain that interact with exogenously administered and/or endogenously produced opiate-like substances.

The consistently observed significant difference between control and methadone offspring and the relative lack of a significant difference in the morphine offspring could suggest that methadone has a different effect than morphine on the developing fetus. We would like to propose, however, that the trends seen in the latencies (control > morphine > methadone) would suggest a similar qualitative response exists and that the differences seen are related to one or more of the following possibilities: (1) a difference in potency of the two drugs (Fig 1); (2) a difference in maternal/fetal pharmacodynamics (hasn't been studied); (3) a difference in effect on selective opiate-receptor interaction in utero as well as at the time of testing.

Our current working hypothesis states that offspring of methadone-treated female rats will exhibit a decrease in the amount of endorphin (β-endorphin, met- and leu-enkephalins) and/or there will be an alteration in the relative amounts (ratio) of these substances. Preliminary studies from our laboratory support the suggestion that specific brain regions in offspring of methadone-treated mothers may be deficient in β-endorphin immunoreactive substances and that synaptosomal preparations of whole brain exhibit an alteration in their interaction with sigma opiate receptor ligand, SKF 10047. Our observations would also suggest a possible decrease in the number, and/or an altered ratio, of the specific opiate receptors (μ, κ, σ) in those areas of the brain associated with the pain process in these offspring.

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