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Prenatal Morphine Enhances Morphine-Conditioned Place Preference in Adult Rats

R. GAGIN, N. KOOK, E. COHEN AND Y. SHAVIT[1]

Department of Psychology, The Hebrew University, Mount Scopus, Jerusalem 91905, Israel

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GAGIN, R., N. KOOK, E. COHEN AND Y. SHAVIT. *Prenatal morphine enhances morphine-conditioned place preference in adult rats.* PHARMACOL BIOCHEM BEHAV **58**(2) 525–528, 1997.—Conditioned place preference (CPP) is a commonly used method for assessing the rewarding qualities of drugs, including opiates. In the present study, we examined long-term effects of prenatal morphine on morphine-associated place preference. Pregnant Fischer 344 rats were given increasing doses of morphine (0.75–12.0 mg/day) in slow-release emulsion during gestational days 12–18. Control rats were injected with vehicle and were fed either with morphine rats or ad libitum. At birth, all litters were culled to 8 pups and fostered to naive dams. Testing began when rats were 10–12 weeks old. Rats prenatally exposed to morphine exhibited a significantly higher preference for the morphine-paired compartment, suggesting that prenatal morphine induces a long-lasting enhancement of its reinforcing effect. Thus, prenatal morphine may result in enhanced activity and/or sensitivity of the endogenous opiate system, thereby placing the organism at higher risk for opiate drug abuse. © 1997 Elsevier Science Inc.

Conditioned place preference Opiates Prenatal morphine Reward

OPIATE substances are well-known for their rewarding properties. Rats will readily self-administer heroin, morphine or other opiate drugs, either systemically or directly, into their brains. Opiate receptors, implicated in drug rewarding effects, are found in brain sites related to the dopamine mesolimbic pathway, the core of the central reward system (1,30,35,36). Opiate activity has been demonstrated both at the origin of the mesolimbic pathway, the ventral tegmental area (VTA), and its termination in the nucleus accumbens (NAC) (2,3,4,28,31).

Drug rewarding influence can be assessed effectively by using the conditioned place preference (CPP) paradigm. This paradigm consists of repeated pairing of a distinctive environment with the rewarding or aversive consequences of the drug being tested. An increase in preference for the drug-paired environment indicates a positively reinforcing effect of the drug. CPP has been demonstrated with opiate drugs and endogenous opiate peptides, administered systemically or intracerebrally (6,26). Conditioning with opiate agonists resulted in a preference for the paired environment (18,19,21,24), whereas opiate antagonists produce conditioned place aversion (19).

Prenatal exposure to opiates can produce long-term changes in the endogenous opiate system. For example, prenatal exposure to opiates enhanced self-administration of cocaine and heroin in adult rodents (23). We recently showed that prenatal morphine enhanced preference for sweet (saccharin) solutions in the adult offspring, which suggests a long-lasting alteration of the reward system (11). Long-term changes in opiate receptors, following prenatal exposure to opiates, have also been reported (9,34,37).

Because opiates can induce CPP and because prenatal exposure to opiates may result in long-term alteration of the reward system, in the present study we examined the effect of prenatal exposure to morphine on morphine-associated CPP in adult rats. Pregnant dams were injected with increasing doses of morphine on days 12–18 of pregnancy. Adult offspring of these dams were tested for morphine CPP.

METHODS

Prenatal treatment

Nulliparous Fischer 344 female rats (Harlan Laboratories, Jerusalem), 10–12 weeks old, weighing 230–250 g, were maintained under standard laboratory conditions (23 ± 1°C; 12-h

[1]To whom requests for reprints should be addressed. E-mail: msshavit@pluto.mscc.huji.ac.il

light–dark cycle, with light on between 19:00 and 7:00). Food and water were always available (unless otherwise specified). To determine the day of estrus, animals were placed with sexually vigorous studs for a brief observation. Estrus was determined by the occurrence of lordosis in response to the stud's sexual approach. Estrous females were housed with Fischer 344 males for approximately 16 h. The day of mating was considered day 0 of pregnancy. Rats mated on the same day were housed in group cages until day 12 of gestation, after which they were separated into individual cages until parturition.

Morphine injections began on day 12 of gestation, because this day just precedes the emergence of opiate receptors in the rat brain (7). The protocol of prenatal morphine administration was based on a pilot study in which we examined different increasing doses of morphine and several schedules of drug administration, including injections up to delivery day. The selected protocol induced analgesia that lasted at least 24 h after the injection and yielded a survival rate of 80% of the newborns.

Pregnant dams were randomly assigned into three groups. The experimental group (Morphine) received increasing doses of morphine HCl (Teva, Israel), 0.75, 1.5, 1.5, 3.0, 6.0, 12.0 and 12.0 mg/injection, on days 12–18 of pregnancy, respectively. Morphine was dissolved in saline and prepared in a slow-release emulsion: morphine–saline solution mixed with light mineral oil (Sigma, Israel) and Arlacel-A (Sigma), in ratios of 8:6:1, respectively (8,10). Each injection was administered subcutaneously (SC) at a volume of 1 ml. Two control groups received daily 1-ml injections of the vehicle emulsion; animals of the ad-lib control group were fed ad libitum; pair-fed control animals were given restricted feeding, corresponding to the average food intake measured on the previous day in morphine-injected dams.

Litters were typically born on days 22–23 of pregnancy; they were culled to 8 pups (with both sexes represented as equally as possible) 24 h after birth and fostered to drug-naive dams. At 3 weeks of age, offspring were weaned, housed in cages of 3–4 rats per cage according to sex and treatment and maintained under standard conditions.

Postnatal testing

Postnatal testing began when offspring were 10–12 weeks old. Sixty-nine male and 62 female rats of the three prenatal treatment groups were tested. Place conditioning was carried out in a dimly lit experimental room at a controlled temperature (23 ± 1°C). Twelve testing boxes were employed simultaneously for conditioning or testing. Each CPP testing apparatus was a 25- × 80-cm plexiglass box, 36 cm high, divided into three compartments: two large, 33-cm-long compartments at both ends of the apparatus and a smaller, 14-cm-long middle compartment that was separated from the other two compartments by sliding (guillotine) doors. The walls of the middle compartment were painted white, and the floor was made of clear plexiglass. The walls of one of the large compartments (designated H) were painted alternating, 2-mm-wide white-and-black horizontal stripes; the walls of the other compartment (designated V) were painted vertical 20-mm-wide white-and-black stripes. The floor of the two large compartments was made of stainless steel rods, 1.5 mm in diameter; in compartment H, the rods were 15 mm apart and laid out perpendicular to the long axis of the testing box; in compartment V, the rods were 9 mm apart and laid out parallel to the long axis of the testing box. To permit monitoring of the cumulative time spent by the animal in each part of the apparatus, each of the three compartments was equipped with an infrared emitting diode and a detector, which were placed on opposite walls, 4 cm above floor level, near the sliding doors. The signal, produced by the detector whenever the animal entered a compartment, was input to a computer and then processed by a dedicated monitoring software.

Procedure

Animals of each prenatal treatment and sex group were assigned randomly into the experimental (morphine) or the control (saline) group. For half the animals (within each group), compartment V was assigned randomly as the conditioned stimulus and compartment H as the neutral environment; for the rest, the conditioned and the neutral compartments were reversed. This procedure was in accordance with an unbiased place conditioning method (6,26). Animals were each assigned to a particular testing apparatus, where they completed all stages of conditioning and testing.

Baseline preference was carried out in all animals during days 1–6 of the experiment. The sliding doors were raised 10 cm above the floor to permit free access to all parts of the apparatus. Each day, animals were placed gently into the middle compartment and allowed 15 min of exploration. The time spent in each compartment was recorded. The average time spent by each animal over the last three sessions in the compartment assigned for morphine conditioning was defined as the baseline preference for this compartment.

Following the completion of baseline measurements, the sliding doors were lowered to confine the animal to a particular compartment. Animals were given four conditioning sessions that were alternated with four sessions of exposure to the "neutral" environment. Prior to each conditioning session (day 8, 10, 12 or 14), the experimental and control animals received injections of either morphine HCl (2.0 mg/kg, SC; this dose was chosen on the basis of previous CPP experiments in our laboratory) or saline, respectively, and were placed for 30 min in the compartment assigned as their respective conditioned stimuli. In neutral-exposure sessions (day 7, 9, 11 or 13), each animal received a saline injection (SC) and was placed for 30 min in its respective neutral compartment.

Postconditioning preference test was carried out on day 15; the sliding doors were raised, and animals were placed in the middle compartment and allowed free access to all parts of the apparatus for 15 min, as in the baseline preference tests. To minimize the variability due to individual differences in baseline preference, the conditioning score was defined as the time spent in the morphine-paired compartment during the postconditioning test minus the baseline preference for the same compartment.

Statistical analysis

Data were analyzed by using a three-way analysis of variance according to prenatal treatment, conditioning group, and sex.

RESULTS

Food intake of morphine-injected dams was reduced to approximately 35% of food consumption by ad-lib dams (3.5–4.0 g/day vs. approximately 10–11 g/day), in accordance with previous reports [e.g. (14)]. There was no difference in the number of newborns among the prenatal treatment groups; average litter size was 8.3 pups. There were no significant differences in neonatal and adult body weight among offspring of the three prenatal treatment groups (Table 1). Newborns of morphine-treated dams did not exhibit any apparent malformation.

Preference conditioning scores are presented in Figure 1. An overall significant preference for the morphine-paired

compartment was observed across all prenatal treatment groups [$F(1,129) = 39.41, p < 0.001$].

Rats prenatally exposed to morphine exhibited a more robust preference for the morphine-paired compartment as opposed to the prenatal control rats. This preference was demonstrated by a significant interaction of prenatal treatment by conditioning treatment [$F(2,129) = 8.109, p < 0.001$].

Further analyses revealed no significant differences between the prenatal pair-fed and ad-lib control groups or between sexes.

DISCUSSION

Morphine exposure during fetal development induced a long-lasting enhancement of the reinforcing effect of morphine. Prenatal morphine was administered with a slow-release emulsion to ensure continuous presence of the drug in the maternal circulation. Alternative procedures in which morphine is dissolved in saline and injected several times a day, involve daily fluctuation in drug levels and recurrent phases of withdrawal between injections. The interpretation of results obtained with such procedures has been questioned (14,17). The present procedure does not simulate perfectly the situation of drug abuse by pregnant women and may be less efficient than intermittent, repeated injections in motivating or rewarding the treated animal. However, we believe that continuous exposure is more appropriate for the investigation of basic processes involved in fetal opiate exposure because it focuses on just one aspect of a complex phenomenon.

The rewarding/reinforcing properties of morphine in the CPP paradigm have been demonstrated in many laboratories [e.g. (5,12,13,20,22,24,32,33)]. Several studies [e.g. (29,32)] have presented a dose–response curve for morphine CPP by showing higher CPP scores associated with larger doses of morphine. Because prenatal morphine rats that were given an identical dose of morphine exhibited higher preference scores than the controls, the perceived reinforcement induced by this dose may have been greater for rats exposed to morphine in utero.

The mechanism underlying the changes in drug-induced reward can be related to changes in the density, affinity or distribution of opiate receptors. Tsang and Ng (34) observed increased met-enkephalin binding in several brain regions following prenatal morphine; Zadina et al. (37) showed increased density and affinity of μ-opiate receptors following prenatal β-endorphin; and Di Giulio et al. (9) observed enhanced development of met-enkephalin-containing neurons in the rat following perinatal morphine. Alternatively, prenatal morphine could affect other neurochemical systems. Both the mesolimbic dopaminergic and the endogenous opiate systems play a key role in morphine reward. Shoaib et al. (29) showed strain differences in the rewarding and dopamine-(DA) releasing effects of morphine in rats. Morphine induced dose-related CPP in two strains of rats, and congruously, both strains showed dose-related DA release in the nucleus accumbens following acute morphine challenge. One strain, however, required much smaller doses of morphine to produce CPP and to increase DA release. Thus, the strain that exhibited higher sensitivity to the rewarding effects of morphine also exhibited higher sensitivity to morphine-induced dopamine release in the nucleus accumbens (29). Prenatal morphine may induce changes in the DA system. For example, the striatal DA system develops subsensitivity following fetal exposure to β-endorphin (25). Following chronic impairment of DA mesolimbic activity, Stinus et al. (31) observed increased reinforcing effects of systemically administered opiates and potentiation of opiate-induced motor activity. Stinus et al. attributed these findings to the development of compensatory supersensitivity in the nucleus accumbens or at higher brain levels. Considering the demonstration that prenatal opiates induce subsensitivity in the striatal DA pathway (25), it would seem plausible to expect a similar effect in the mesolimbic system following prenatal morphine. Such subsensitivity may in turn induce compensatory supersensitivity of the endogenous opiate system, thus rendering exogenous morphine more reinforcing.

Other possible mechanisms include changes in secondary messengers, such as G-protein-cAMP, in the nucleus accumbens, as demonstrated in opiate-dependent individuals (27) or upregulation/supersensitivity of NMDA receptors due to blockade of these receptors by morphine (16).

We recently reported that prenatal exposure to morphine induced long-term alterations in opiate-induced analgesia and in reward processes (11). Adult rats prenatally exposed to morphine exhibited elevated analgesic scores in response to acute morphine challenge and increased preference for sweet (saccharin) solutions. These data and the present findings suggest that prenatal morphine produces a long-term increase in

TABLE 1

BODY WEIGHT (g) OF NEWBORN AND ADULT RATS OF THE THREE PRENATAL TREATMENT GROUPS

Prenatal Treatment	Newborns*	Adult Males	Adult Females
ad libitum ± SEM	8.94 ± 2.16	324 ± 12.58	164 ± 4.76
n	22	24	23
Pair-fed ± SEM	8.51 ± 1.76	317 ± 12.15	172 ± 5.70
n	21	24	24
Morphine ± SEM	8.12 ± 2.67	325 ± 11.01	160 ± 4.45
n	16	24	24

*No significant difference in body weight were detected at birth between the sexes.

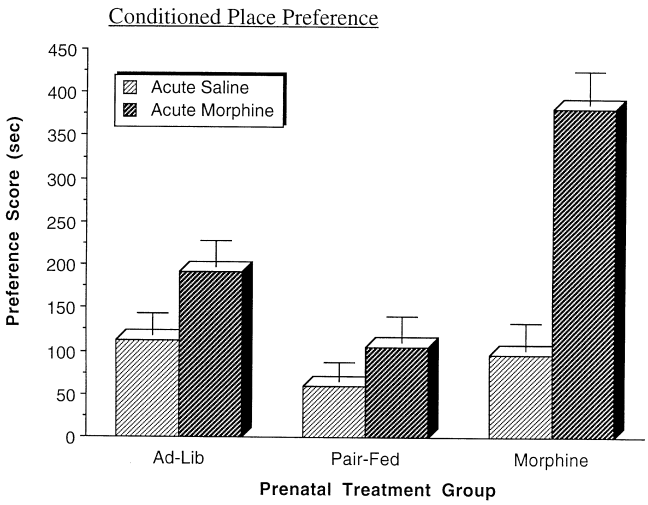

FIG. 1. Preference scores, defined as time in the conditioned compartment during test minus time in the same compartment during baseline, in female and male rats prenatally treated with morphine (Morphine; $n = 44$), vehicle and pair-feeding (Pair-Fed; $n = 44$) or vehicle and ad libitum feeding (Ad-Lib; $n = 43$). For half the rats in each prenatal treatment group, the conditioned compartment was associated with morphine (2 mg/kg; Acute Morphine); for the other half, the conditioned compartment was associated with saline (Acute Saline). All data are expressed as means ± SEM.

the activity/sensitivity of the endogenous opiate system, which is reflected in enhanced sensitivity to acute morphine challenge and in a highly reactive reward system. Accumulating evidence suggests that the positive reinforcing effects caused by activation of the endogenous reward pathways determine the abuse potential of certain drugs and initiate the addictive process (15). The rewarding properties of different substances, as demonstrated by the choice of behavior of animals in the CPP paradigm, may underlie their potential for abuse (36). In the present study, prenatal morphine rats exhibited significantly higher preference scores as compared to control rats in response to the same dose of morphine, suggesting that they perceive this dose as more rewarding/reinforcing. Thus, prenatal opiates may increase the abuse potential of certain (opiate) drugs and the risk for drug addiction.

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