PHARMACOLOGY AND TOXICOLOGY

Increased Anxiety and Reduced Pain Sensitivity in Offspring of Rats after Prenatal Morphinization

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Administration of morphine to outbred female rats is shown to result in increased anxiety in the conflict test and reduced pain sensitivity in the tail flick test in 2.5-month-old offspring. In prenatally morphinized offspring the analgetic effect of morphine was enhanced, while the anxiolytic effect of buspirone was lower than in intact animals, which suggests rearrangements in the opioid and serotoninergic systems of the brain.

Key Words: *morphine; offspring; anxiety*

Narcotics abuse is becoming more and more widespread. Of particular concern is the growing addiction among women in many countries [2,8,11]. Numerous investigations have established adverse effects of narcotics on pregnancy and fetal development. The majority of authorities report the withdrawal syndrome in newborns [4,6,10,15] and high prenatal mortality [12], as well as various congenital abnormalities [6,9]. However, the long-term consequences of the prenatal effect of narcotics on the fetus are still poorly understood.

The aim of our study was to investigate the anxiogenic behavior and pain sensitivity of rats exposed to morphine *in utero*.

MATERIALS AND METHODS

The experiments were performed on outbred male rats, whose mothers had received intraperitoneal injections of morphine (10 mg/kg, twice a day) from the 9th to 19th day of gestation. The mothers of control rats were injected with isotonic NaCl solution during the same period. The 2.5-, 4-, and 5-month-old rat pups were tested in a conflict situation as described earlier [14] with modifications [3]. In brief, after a 48-hour water deprivation the animals were daily during 2 days placed for 10 min in a setup where they were allowed to drink water from a dish. On the third day, 10 sec after the first water intake an electrical current (0.5 mA) was applied to the setup and the number of punishable water intakes, accompanied by shocking, was automatically recorded. In 2.5-month-old animals conflict behavior was also recorded against the background of phenazepam (1 mg/kg) and buspirone (5 mg/kg) injected intraperitoneally 30 min before the test. The pain sensitivity of 2.5- and 4-month-old rats was assessed in the tail flick test by measuring the latency of tail withdrawal in response to thermal stimulation. The analgetic effect of morphine (5 mg/kg, i.p. 30 min before the experiment) was studied in 2.5-month-old rats using the same test. The statistical processing of the results was performed using the Student t test [5].

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RESULTS

Prenatal morphinization markedly disturbs the conflict behavior of animals, which is most pronounced in young individuals. The number of punishable water intakes was considerably lower (4.7-fold) in offspring of morphinized rats in comparison with controls, which suggests an increased anxiety in these animals (Fig. 1).

The conflict behavior of animals changes markedly with age: the number of punishable water intakes drops and the anxiety rises. The dynamics of this parameter in prenatally morphinized rats is completely different. The comparison of this parameter in 2.5-, 4-, and 5-month-old rats reveals its rise in 4-month-old rats with a subsequent return to the initial level at 5 month, i.e., the index of anxiogenicity in 2.5- and 5-month-old rats is at the same level (Fig. 1).

Phenazepam (1 mg/kg) produced a pronounced anxiolytic effect in both experimental and control rats (Table 1). On the other hand, buspirone (5 mg/kg) increased the number of punishable water intakes in prenatally morphinized rats to lesser extent. Although buspirone reliably reduced anxiogenicity in morphinized rats in comparison with the background level, the anxiety level still surpassed that in the controls and remained elevated (Table 1). This effect of buspirone in experimental animals is probably related to changes in the serotoninergic system in offspring of mothers receiving morphine during pregnancy. This is confirmed by the increased number of binding sites and reduced affinity of 5-HT1 receptors to the ligand in 2-month-old offspring of rats receiving morphine for a long time in comparison with controls [1].

The study of pain sensitivity revealed that prenatal treatment with morphine results in a marked decrease of pain sensitivity of 2.5-month-old animals in response to thermal stimulation (Fig. 2). However, in 4-month-old rats no reliable differences were observed between the experimental and

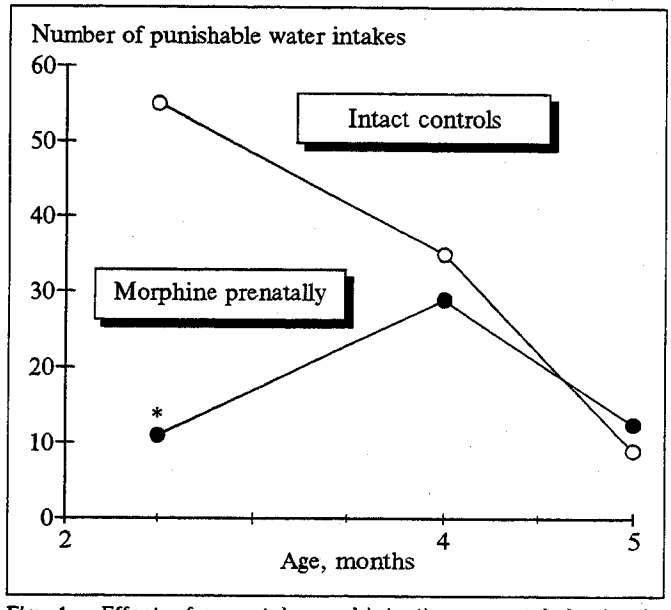

Fig. 1. Effect of prenatal morphinization on rat behavior in "conflict situation" test. Asterisk denotes $p<0.01$.

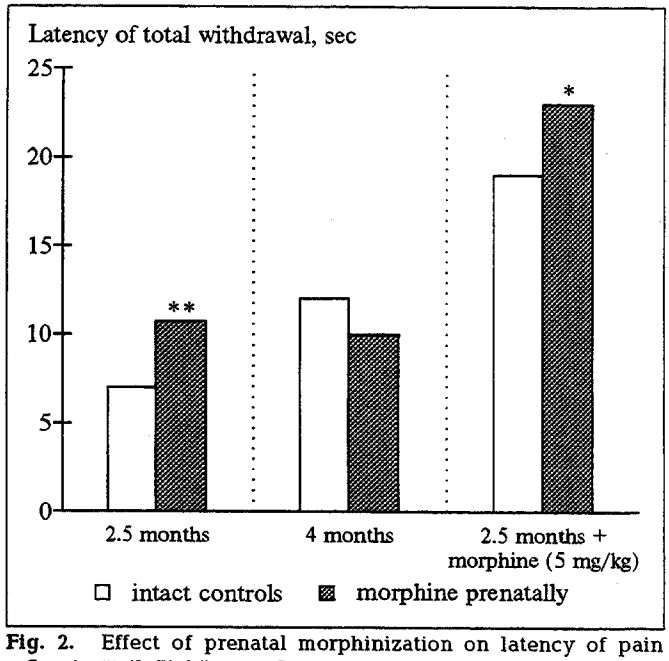

Fig. 2. Effect of prenatal morphinization on latency of pain reflex in "tail flick" test. One and two asterisks denote $p<0.05$ and $p<0.01$, respectively.

TABLE 1. Effect of Prenatal Morphinization on Behavior of 2.5-Month-Old Rat Offspring ($n=20$) in Conflict Situation Test against the Background of Phenazepam and Buspirone

Group	Initial number of punishable water intakes without tranquilizers	Phenazepam, 5 mg/kg	Buspirone, 5 mg/kg
Intact controls	56±15	365±35**	74±12
Morphine prenatally	12±4*	347±36**++	36±10+

Note: One and two asterisks denote reliability of differences in comparison with intact controls without tranquilizers for $p<0.01$ and $p<0.001$, respectively, and one and two crosses, in comparison with initial value of index (morphine prenatally) for $p<0.05$ and $p<0.001$, respectively.

control groups. In prenatally morphinized animals morphine (5 mg/kg) produced a reliably higher analgetic effect than in the controls. The findings on the alteration of pain sensitivity in offspring caused by prenatal morphinization are in conformity with a previous report [7] on a reduced sensitivity to thermal stimulation under conditions of the "hot plate" test in 80-90-day-old rats prenatally exposed to morphine. The observed changes in pain sensitivity are apparently due to changes in the opioid system of prenatally morphinized offspring. This is confirmed by the fact that administration of morphine to female rats throughout pregnancy results in an increased number of opioid receptors in the brain of newborn offspring followed by its drop below the control value with age [13].

Thus, the observed increased anxiogenicity and reduced pain sensitivity in prenatally morphinized rats together with reliable changes in their reaction to morphine and buspirone suggest rearrangements of the opioid and serotoninergic systems of the brain in offspring of rats exposed to morphine during pregnancy.

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