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BRIEF COMMUNICATION

The Effects of Prenatal Morphine on the Responsiveness to Morphine and Amphetamine

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WAGNER, G C , M F JARVIS, D S GOTTESFELD, M GIORLANDO AND J RABII *The effects of prenatal morphine on the responsiveness to morphine and amphetamine* PHARMACOL BIOCHEM BEHAV **24**(3) 757–759, 1986 —The effects of morphine administered to pregnant Sprague-Dawley rats on the schedule-controlled behavior of the offspring were examined It was observed that both male and female adult rats exposed prenatally to morphine were tolerant to the disruptive effects of morphine on fixed-interval responding compared to age-matched controls These morphine-treated rats, however, were neither tolerant nor supersensitive to the disruptive effects of the catecholaminergic agonist, amphetamine, and did not exhibit any alteration in their steady state levels of central monoamines These observations are discussed in relation to the effects of prenatal morphine exposure on unconditioned behaviors

Prenatal morphine Fixed-interval schedule Amphetamine Monoamines

RATS treated prenatally with morphine exhibit pronounced physiological and behavioral deficiencies (for a recently compiled literature review see [12]) However, despite extensive investigation of other aspects of this syndrome, there have been few, if any, studies conducted on the responsiveness of adult rats which have been prenatally exposed to morphine to a subsequent, acute administration of morphine in a schedule-controlled behavioral paradigm Accordingly, the present study was designed to determine if adult rats which have been prenatally exposed to morphine exhibit either a tolerance or a sensitization to the effect of morphine on operant responding upon its readministration In addition, since the endogenous opiates have been linked to the central catecholaminergic systems [2,5], the responsiveness of these prenatally-treated rats to a catecholaminergic agonist (amphetamine) as well as their central monoamine levels were examined

METHOD

Subjects

Sprague-Dawley female rats (Charles River) were housed two per cage in standard pan cages and maintained on a 14 hr light/10 hr dark cycle (lights on at 0500) Sexually experienced male rats were introduced to each cage and once mating had occurred, the female rats were transferred to individual cages The pregnant female rats were randomly assigned to one of two groups (1) a morphine sulfate (MS)-treated experimental group, or (2) a saline (S)-treated control group The experimental group was injected twice on day five post-conception (once at 0730 and once at 1630) with 5 0 mg/kg MS (Merck & Co) subcutaneously On day six they were injected with 5 0 mg/kg MS in the morning and 10 0 mg/kg MS in the evening On day seven and thereafter through day twelve, they received two daily injections of 10 0 mg/kg MS The control group of females received two injections of 0 9% NaCl on days five through twelve These subjects were derived from an ongoing series of studies involving prenatal MS treatment Gestational and birth statistics have been presented elsewhere [8]

The offspring of each group were culled to eight per litter and then left undisturbed until weaning at 21 days of age As in previously reported studies [7, 8, 11], cross-fostering was not incorporated into the design of the present study They were then housed individually in suspended metal cages and maintained on a 12/12 light/dark cycle with lights on at 0700 Purina rat chow and water were freely available Training on the fixed-interval schedule was started when the rats were approximately 120 days old At that time the offspring were divided into four groups experimental male (n=6) and female (n=5) rats born to MS-treated mothers and control male (n=6) and female (n=5) rats born to S-treated mothers

Apparatus

Experimental sessions were conducted in an operant box

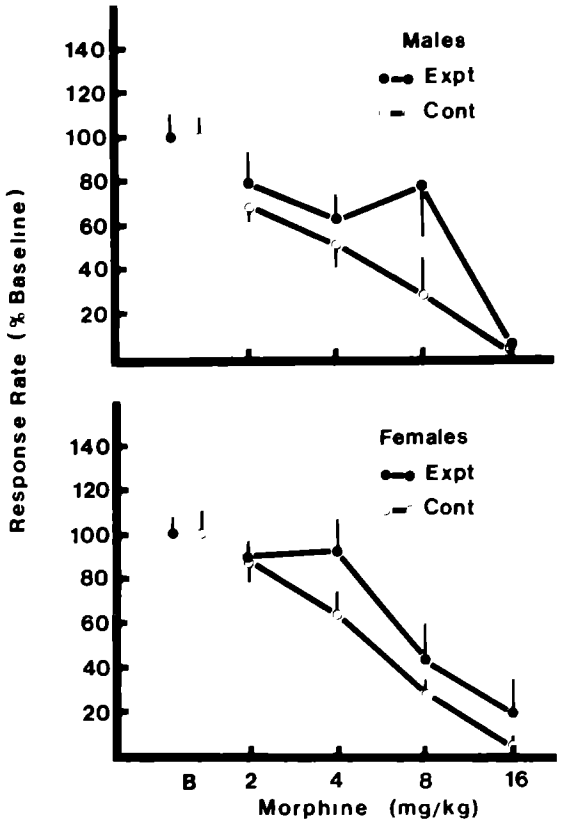

FIG 1 Response rate as a function of morphine dose for adult rats treated prenatally with morphine (closed circles) or saline (open circles) Percent baseline refers to the ratio of drug response rate to control response rate Rats received IP morphine 10 min prior to the start of the FI session Vertical bars=SEM

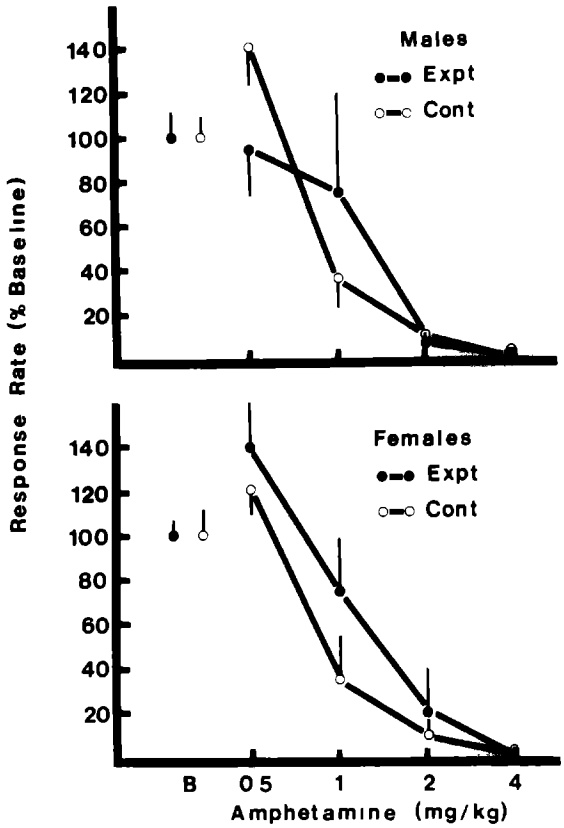

FIG 2 Response rate as a function of amphetamine dose for adult rats treated prenatally with morphine (closed circles) or saline (open circles) Percent baseline refers to the ratio of drug response rate to control response rate Rats received IP amphetamine 15 min prior to the FI session Vertical bars=SEM

(51×33×48 cm) enclosed in a ventilated, sound attenuating chamber The manipulandum was a Gerbrands model G6312 lever mounted 12 cm above the floor and 6 cm below a GE 1813 session light A water dipper (which delivered 0 1 ml tap water) was located 4 cm to the right of the lever and 0 5 cm above the floor Animals had free access to food and were water deprived 23 25 hr/day The experimental session was 30 min The rats were allowed 15 min access to water beginning 15 min after the session

Procedure

Rats were first trained to lever press for water reinforcement on a continuous reinforcement schedule When animals were reliably responding, they were shifted to a fixed-interval 90s (FI-90) schedule of reinforcement When responding on the FI-90 schedule had become stable, 0 9% saline or MS was administered IP 20 min prior to the session The doses of MS were varied in a random manner until a complete dose-response curve was obtained The MS (or saline) was administered no more often than every third day and only after a stable baseline was achieved Sessions were conducted seven days/week When the MS dose-response curve was completed, the entire procedure was repeated with amphetamine (Sigma Chemical Co) The amphetamine was administered IP 15 min before the session Following the MS and the amphetamine dose-response curve determinations, rats were sacrificed and neurochemical assays were conducted according to the method of Wagner *et al* [9,10]

RESULTS

Comparisons of baseline response rates between the experimental and control rats revealed slight but nonsignificant differences Therefore, all drug effects were evaluated using analysis of covariance of drug response rate with baseline responding serving as the covariate [1] In all four groups morphine produced a dose-dependent decrease in response rate (Fig 1) The response rate ED-50 of the prenatal morphine-treated rats (8 86±1 56 mg/kg) was significantly greater than that of the prenatal saline-treated rats (4 79±0 58 mg/kg) (Student's t-test, $p<0$ 05) Analysis of covariance reveals a significant treatment × dose interaction, $F(3,26)=3$ 47, $p<0$ 05, between the experimental and control males Subsequent post hoc analysis via Fisher's LSD test ($p<0$ 05) revealed significant differences between the experimental and control males at morphine 4 0 and 8 0 mg/kg There were no significant differences for treatment or treatment × dose interactions between the experimental and control females

In three of the four groups, amphetamine produced a biphasic effect on response rate with the lowest dose increasing and the higher doses decreasing responding (Fig 2) The experimental males, however, exhibited only a dose-

dependent decrease in responding Analysis of covariance revealed no significant differences between the experimental and control groups for the main effect of treatment or treatment × dose interactions Two of the experimental males died before completion of the amphetamine dose response determination and were therefore excluded from the analysis of the amphetamine data

Two weeks after the last drug administration all rats were sacrificed and brains removed for monoamine assay Prenatal morphine treatment did not produce any (permanent) changes in central monoamine content in any of the regions assayed compared to the saline treated controls with one exception the experimental female rats had significantly lower telencephalic dopamine levels than the control females (data not shown)

DISCUSSION

Prenatal exposure to opiates has been shown to (1) directly affect a variety of developmental processes such as growth rate [3,6] and vaginal opening [7], (2) alter unconditioned behaviors such as sexual behavior [7] and locomotor activity [5], and (3) result in a shift in the analgesic effects of morphine and morphine-like agents [6] The present study was designed to evaluate the effects of prenatal morphine treatment on responding maintained by a fixed-interval schedule of water delivery in the adult offspring It was observed that prenatal morphine-treated rats exhibited a significant tolerance to the disruptive effects of morphine This tolerance was evident in the males but not in the female subjects

The endogenous opiates have been linked to the central catecholamine systems [2,4] and prenatal opiate exposure has been shown to reduce the uptake of labeled dopamine and norepinephrine [3] and cause a depletion of central dopamine and norepinephrine [5] However, it should be noted that, as in the present study, Rech *et al* [5] found that the depletion of central catecholamine steady state levels which they observed in young offspring, was no longer apparent in 90 day old rats The lack of an effect of the prenatal morphine treatment on the sensitivity to the disruptive effects of amphetamine on operant responding in these adult rats is consistent with this latter observation

In summary, prenatal exposure to morphine was found to produce a tolerance to the response rate-disruptive effects of acutely administered morphine in the adult offspring These prenatally treated rats had normal central catecholamine levels and were equally sensitive to the disruptive effects of the catecholaminergic agonist, amphetamine, on operant responding as compared to control rats

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