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BRIEF COMMUNICATION

Phenobarbital During Pregnancy Alters Operant Behavior of Offspring in C57BL/6J Mice[1]

LAWRENCE D. MIDDAUGH, CARROLL A. SANTOS, III AND JOHN W. ZEMP

Departments of Biochemistry, and Psychiatry and Behavioral Science
Medical University of South Carolina, Charleston, SC 29401

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MIDDAUGH, L. D., C. A. SANTOS, III, AND J. W. ZEMP. *Phenobarbital during pregnancy alters operant behavior of offspring in C57BL/6J mice.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1137–1139, 1975. – Offspring of C57BL/6J injected daily with phenobarbital for the last third of pregnancy responded less than control animals when maintained on various fixed ratio schedules of reinforcement. The response decrement became more pronounced as the schedule demands were increased and was noted in offspring of both sexes. The highest dose (80 mg/kg) was less effective than the 2 lower doses (20 mg and 40 mg/kg) in producing the decrement which may reflect a selection factor due to high neonatal mortality previously reported at this dose. The study provides no evidence of the mechanism mediating the long term behavioral abnormality but does clearly extend the finding of such changes to doses which do not produce increased neonatal mortality or noticeable morphological changes.

Prenatal maternal phenobarbital Operant behavior in C57BL/6J mice

PHENOBARBITAL is being ingested by a substantial number of pregnant women. Epidemiological studies suggest that from 24 to 32 percent of pregnant women ingest some type of sedative, frequently phenobarbital, generally during the last trimester [4,5]. There are also a number of women with epilepsy using phenobarbital as an anticonvulsant throughout pregnancy [3,5]. In addition, the drug is used by members of the drug subculture including use during pregnancy [3]. Finally, phenobarbital has been administered to gravid women for 2 week periods prior to parturition as a prophylaxis for neonatal hyperbilirubinemia [16]. In spite of the frequent use of phenobarbital during pregnancy, there is relatively little information regarding potential effects of the drug on offspring, particularly long-term effects. Although administering the drug to pregnant women as a prophylaxis for neonatal hyperbilirubinemia has been reported to produce no detrimental side effects [16], a thorough investigation for possible effects is absent [15]. Withdrawal symptoms have been noted in neonates addicted to the drug during pregnancy [3] and high doses of phenobarbital injected into pregnant rats can increase fetal death and produce morphological abnormalities in surviving offspring [8].

There is also some evidence that mature offspring of rats [1,7] and of mice [9, 10, 17] injected with the drug during pregnancy have altered brain function as evidenced by behavior abnormalities.

We have previously reported the results of experiments on offspring of C57BL/6J mice injected daily with phenobarbital for the last third of pregnancy [9, 10, 17]. Increased neonatal mortality and reduced body weight in mature surviving offspring were noted for animals injected with either 80 mg or 40 mg/kg of the drug [17]. A 20 mg/kg dose had no significant effect on these measures. The high dose of the drug produced reductions in offspring brain weights accompanied by reduced levels of nucleic acids and protein. We have also reported that offspring of mice injected with a 40 mg/kg dose of the drug for the same time period were retarded in the development of several motor reflexes [9] and differed from controls on 3 behavioral tests conducted after maturity [10]. A comprehensive study varying drug dose and assessing the behavioral affects on mature offspring of both sexes, however, is currently absent from the literature.

The purpose of the study reported here was to assess the effects of phenobarbital (20–, 40–, and 80 mg/kg) injected into pregnant mice for the last third of pregnancy on lever responding by offspring maintained on increasing fixed ratio schedules of reinforcement. This task was previously effective in differentiating female offspring of animals injected with 40 mg/kg phenobarbital from control mice.

METHOD

Animals for the study were C57BL/6J mice (*Mus*

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musculus) obtained from our breeder colony. They were maintained in a temperature regulated room (23° ± 2°C) on 12 hr light:dark cycle and had food and water available ad lib until testing which required food deprivation. Pregnancies were determined by inspection for sperm plugs each morning and were verified by weight gains (≥4 g) 12 days after plug detection. Animals in the drug groups were injected subcutaneously each morning with 20–, 40–, or 80 mg phenobarbital sodium per kg bodyweight (P_{20}, P_{40}, P_{80}) for the last 6 or 7 days of pregnancy. Saline control (SC) animals were injected for the same time period with 0.9 percent saline. In each case, the animal was injected with a 0.008 ml solution/g body weight. On the day of delivery, the litter was culled to 6 and offspring were reared by their biological mother until weaning at 21 days of age. After weaning, animals were caged according to sex with 2–6 animals per cage until behavioral assessment when they were maintained in individual cages.

At 90 days of age, the behavior of the pups from the various treatments was assessed using an operant task requiring the animal to make an increasing number of responses per unit of reinforcement across days. The animals were tested in 1 of 6 operant chambers enclosed in sound attenuated boxes. The chambers (16 × 16 × 11.4 cm) were constructed of Plexiglas with stainless steel grid floors. A food tray with a 1.9 × 2.5 cm opening was centrally located on one wall at floor level. A Lehigh Valley Model No. 121–03 rodent lever located 4 cm to one side of the food tray and 3.0 cm above the floor served as the response indicator. Eight grams dead weight on the lever activated a microswitch which initiated programming equipment. After one week on a food deprivation schedule which reduced body weight to 75–85 percent of ad lib levels, the animals were familiarized with the test chamber and self-trained to press the lever for food reinforcement (20mg Noyes pellets). After this, animals were run 30 min per day for 5 days on each of the following schedules of reinforcement: CRF (1 response per reinforcement), FR5 (5 responses per reinforcement), FR20, and FR40. Male animals were additionally run three days each on FR80, FR120, and FR160.

RESULTS

Injecting pregnant mice with phenobarbital did not alter the ability of offspring to acquire the lever response producing food reinforcement. Approximately 90 percent of the animals in each group acquired the response to a criterion of ≥ 10 responses per session during two 15 min self shaping sessions. The remaining animals met this criterion after two additional 30 min sessions.

The effect of these treatments on behavior maintained by the various fixed ratio schedules of reinforcement was assessed by comparing the drug groups of each sex with their respective SC groups. Mean responses generated per daily session under each schedule of reinforcement (i.e., over a 5 day test period) were calculated for each animal and served as an index of its performance on the particular schedule. Since there was a great deal of individual variation within each group, particularly on the higher fixed ratio schedules, group tendencies are reported as medians. These data are summarized in Fig. 1.

Control animals showed the expected increased responding with increasing demands of the reinforcement schedule. For males, statistical comparison of individual

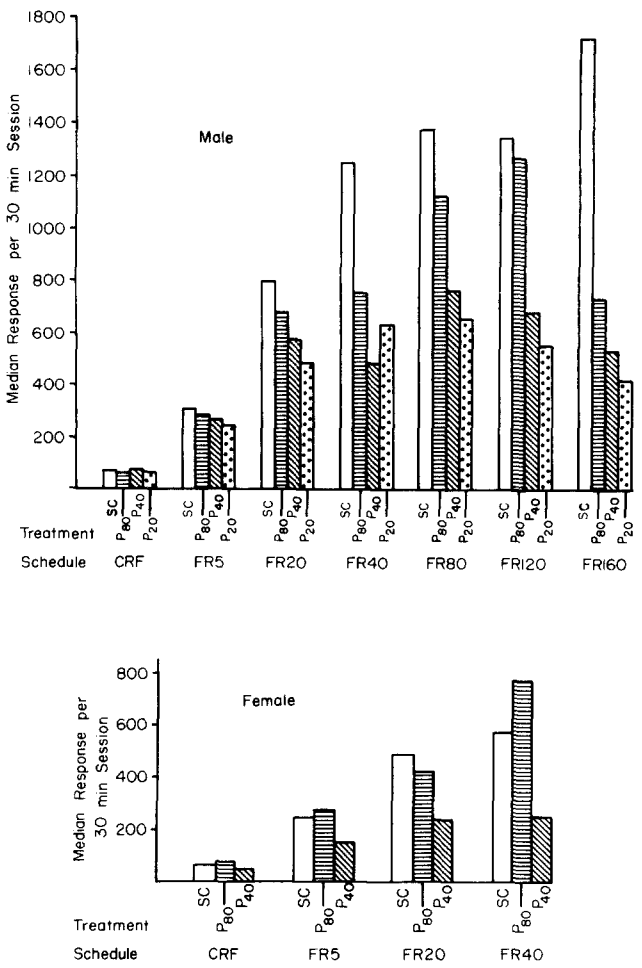

FIG. 1. Effect of phenobarbital during pregnancy on lever responding of mature offspring maintained under various fixed ratio (FR) schedules of reinforcement. Animals were 90-day-old offspring of C57BL/6J mice injected daily with saline (SC) or phenobarbital at doses of 20 mg (P_{20}), 40 mg (P_{40}), or 80 mg (P_{80}) per kg body weight for the last third of pregnancy. See text for statistical analysis. The bars represent medians determined from Ns of 6–11 per group. Upper and lower graphs summarize performance of male and female offspring respectively.

drug groups with saline controls via Mann-Whitney U-Tests [13] established significant reductions in responding at the 95 percent level of confidence for the P_{40} and P_{20} groups beginning at FR40 and extending through the highest schedule tested. The median score for the P_{80} group does not differ significantly from that of the SC group until FR 160.

The pattern of responding for SC females was similar to that of males but at a reduced level. Female animals of the P_{80} group responded at rates somewhat higher than the SC group on all schedules tested, however, none of these differences are significant. A significant reduction in responding by the P_{40} group compared to either the SC or P_{80} groups was noted for all schedules tested except CRF.

DISCUSSION

This study clearly establishes that injecting mice with

phenobarbital for the last third of pregnancy produced long lasting behavioral changes in offspring of both sexes. In this study, responding maintained by various fixed ratio schedules of reinforcement was reduced. The reduction became more pronounced as the animal was required to increase its response output per unit of reinforcement (i.e., on the higher ratio schedules). We have previously reported [17] that the two higher doses of phenobarbital (80 mg and 40 mg/kg) caused a weight reduction in mature surviving offspring and it could be argued that the reduced weight might contribute to the reduction in response output due to the increased effort required of the lighter animals to depress the lever. This however, is certainly not a complete explanation since the highest dose produced the greatest weight reduction [17] yet in the current study had the least effect on response output. In addition, female offspring of animals injected with 40 mg/kg of phenobarbital have previously been reported to show a response decrement on a similar task differing only in that the response was merely contact with a metal disc which presumably would be independent of body weight [10]. In the same study, it was also shown that the response decrement was due to the schedule and not to the length of time on food deprivation.

The reduced effect of the higher drug dose in the current study was certainly unexpected and we currently have no explanation for this finding. Reverse dose-response effects have been reported in other studies on the effects of maternally administered amphetamine or morphine on offspring [2, 12, 17]. It should also be noted that the 80 mg/kg dose of phenobarbital produces a large increase in neonatal mortality [17] hence, selection factor might account for the reduced behavioral effect noted in surviving offspring. In addition, we have found that 80 mg/kg of this drug injected into adult animals produced behavioral effects different from that of the two lower doses used in this experiment (L. D. Middaugh, unpublished observations). Whereas the 80 mg/kg dose depressed responding maintained by a variable interval schedule of reinforcement, 20 mg and 40 mg/kg doses produced an increased response output. It is thus probable that the different doses are producing different physiological effects on either the pregnant animal or her fetuses.

The mechanism mediating these long-term behavioral changes is currently unknown. Possibly, changes in mother-infant interaction essential for normal development [6] may have contributed to the observed behavioral changes in adult offspring. Either abrupt withdrawal from the drug following parturition or the previously reported [17] changes in neonates of animals injected with the drug would be capable of altering this interaction.

It is also known that phenobarbital can cross the placenta in both animals [14] and humans [11]; and is reported to be highly concentrated in fetal liver, adrenal, and brain stem tissue. Hence, it is possible that the drug may be interfering with the development of the brain and/or pituitary adrenal system to produce the long-term behavioral effects.

Although this study provides no information regarding the mechanism by which phenobarbital injected into pregnant animals produces the long lasting changes in offspring behavior, it does clearly establish the existence of such alterations and should prompt further investigation of the extent and mechanism of the change.

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