RAPID COMMUNICATION

Effects of Perinatal Diazepam Administration on Two Sexually Dimorphic Nonreproductive Behaviors

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GUILLAMÓN, A., J. M. CALÉS, M. RODRIGUEZ-ZAFRA, C. PEREZ-LASO, A. CAMINERO, Mª. A. P. IZQUIERDO AND S. SEGOVIA. Effects of perinatal diazepam administration on two sexually dimorphic nonreproductive behaviors. BRAIN RES BULL 25(6) 913-916, 1990. — The effects of prenatal and/or early postnatal diazepam (DZ) administration on open field activity and continuously reinforced lever-pressing response were studied. Rat pups of both sexes were prenatally (during the last week of pregnancy) and/or postnatally (from the day of birth to day 16) daily exposed to a 2.5 mg/kg dose of DZ. At the age of 60 days all groups were tested in the open field for 5 consecutive days and thirty days later they were studied in a continuously reinforced lever-pressing situation during four consecutive days. In the open field test, females showed greater activity than males and prenatal and/or early postnatal DZ treatments did not alter this sexual dimorphism, although all treatments decreased the open field activity in both male and female 60-day-old rats. In the Skinner box, 90-day-old males presented higher rates of lever-pressing response than females, and only the early postnatal DZ treatment was effective in altering this sexual dimorphism, by decreasing the male’s but not female’s rates of response. These results are discussed in regard to the possible interaction between DZ and gonadal hormones during the early sexual differentiation period.

Diazepam Sex differences Activity Learning Operant conditioning Rat

THE long-term effects of perinatal exposure to benzodiazepines on the later behavior of rats have been repeatedly studied (8, 14, 22). Lyuvimov et al. (16) demonstrated impaired acquisition of a conditional avoidance response in rats exposed to diazepam (DZ) throughout gestation. Later, Kellog et al. (15) reported that prenatal administration of DZ during the last week of pregnancy effectively interfered with the development of arousal processes in the offsprings by suppressing the development pattern of locomotor activity and acoustic startle response that normally take place in the third postnatal week. Similar long-term behavioral alterations after perinatal DZ exposure have also been found on other nonreproductive behaviors, such as open field performance or acquisition and retention of a simultaneous brightness-discrimination task using a complex six-unit maze (5,6). These studies have indicated that such early and permanent effects of DZ depend upon the timing in which DZ is administered and the type of behavior. Thus, in relation to the open field test, it has been found that postnatal but not prenatal DZ-exposed rats show more number of squares crossed than control animals (5,6), whereas only the prenatal DZ treatment is effective in reducing rearings at 35 days of age (6). More interesting behavioral results concern the learning performance of DZ-exposed animals during perinatal period. Gai and Grimm (6) found an acquisition and retention deficit of prenatally DZ-exposed rats when they were tested in a complex simultaneous-choice discrimination maze but not when the task consisted of successive discrimination learning in two parallel straight alleys. In addition, Gai and Grimm demonstrated that such deficits in acquisition and retention were dose-dependent (6). Similar results were reported by Frieder et al. (5) in both prenatal and early postnatal DZ-treated rats, indicating that the prenatal effects of DZ on later acquisition and retention of a complex simultaneous discrimination task are restricted to the third week of pregnancy.

Simultaneous and successive discrimination tasks involve a response inhibition process and taking all these results into account, it appears that, at least when the solution of the task requires a

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were no significant differences between Nt and Veh groups (Tukey’s test: \( p > 0.05 \)), all DZ treatments were effective in reducing activity as compared with the Nt or Veh rats.

**Learning**

Figure 2 shows the rate of response obtained in the different groups used in the present experiment. The analysis of variance revealed sex differences, with males presenting higher rates than female rats (males: 11.85, females: 9.59; Sex: F(1,83) = 58.62, \( p < 0.0001 \)). Moreover, a Treatment difference and a Sex \( \times \) Treatment significant interaction were found [Treatment: F(4,83) = 8.71, \( p < 0.0001 \); Sex \( \times \) Treatment: F(4,83) = 5.14, \( p = 0.0009 \)]. However, Sex did not interact in a significant manner with the Treatment and Days variables, F(12,249) = 1.05, \( p > 0.05 \), although learning improved in all groups through the different acquisition sessions [Days: F(3,249) = 9.71, \( p > 0.0001 \)]. As can be observed in Fig. 2, Nt- and Veh-males showed higher number of responses per minute than Nt- and Veh-females (all comparisons, \( p = 0.01 \)) and no differences between Nt- and Veh-males or between Nt- and Veh-females were found (\( p > 0.05 \) in the two cases). Prenatal DZ treatment affected neither males’ nor females’ performance (Pre-males vs. Nt-males: \( p > 0.01 \); Pre-males vs. Veh-males: \( p > 0.01 \); Pre-females vs. Nt-females: \( p > 0.01 \); Pre-females vs. Veh-females: \( p = 0.1 \)). Although early postnatal or pre- plus postnatal DZ treatments impaired the acquisition in males as compared with the control male groups (Post-males vs. Nt-males: \( p = 0.01 \); Post-males vs. Veh-males: \( p = 0.01 \); PP-males vs. Nt-males: \( p = 0.05 \); PP-males vs. Veh-males: \( p = 0.05 \)), decreasing their rates of response at the levels of that showed by females (\( p = 0.1 \) in all comparisons with respect to all female groups). By contrast, no statistical differences between Post-females and Nt- or Veh-females or between PP-females and Nt-females or PP-females and Veh-females were found (all \( p > 0.1 \)).

**DISCUSSION**

The present results show a clear sexual dimorphism in both open field activity (females showed more number of squares crossed than males) and free-operant CRF situation (males presented more responses per minute than female rats). In relation to the CRF schedule, DZ decreased the males’ response rate in the Post group at the levels shown by females. A similar effect was observed in the PP-males, which could be explained as a consequence of the DZ postnatal administration, since prenatal DZ treatment had no effect on either sex. By contrast, DZ treatment did not affect differentially males’ and females’ activity in the open field test, although prenatal and/or postnatal DZ reduced activity in both sexes.

It is well demonstrated that female rats are more exploratory and present higher locomotor activity than males (1, 2, 7, 10, 13, 17). Therefore, differences in performance between males and females in learning situations have been frequently explained on the basis of the greater exploratory behavior and/or activity levels shown by female rats (2, 12, 23, 24), and sex differences found under CRF schedule have not been an exception (23). According to this explanation, the sexual dimorphism obtained in the acquisition in the present experiment could be the result of the higher tendency of female rats to develop activities other than lever pressing. However, this explanation cannot be applied to our results, because prenatal DZ administration had a different effect on the open field activity than on the CRF performance. On the other hand, undernutrition has been seen as a side effect of the administration of sedative drugs in teratological studies (22). It could explain the long-lasting deficits observed in the perinatally treated pups. However, had the early postnatal DZ treatment borne undernutrition as a side effect, then DZ-treated male as well as female rats should have suffered the same deficit in performance, but our results show that CRF performance was affected in a sex-dependent manner.

In relation to the long-term effects of DZ on open field activity, our results markedly differ from those previously found by other authors. In previous studies (5,9) postnatal but not prenatal DZ treatment resulted in consistent and lasting hyperactivity in 35-day-old rats, and only females were affected differentially by a large dose (20 mg/kg), which reduced the number of squares crossed to the level of males. This discrepancy could be interpreted on the basis of the different age at which the animals were tested (35 days in these reports vs. 60 days in the present study).

With respect to the long-lasting effects of DZ on learning situations, it has been shown that in a simultaneous discrimination situation, male and female rats present a different vulnerability to the perinatal DZ administration (5,9); whereas prenatally administered DZ impairs maze learning in males but not females, postnatal DZ seems to affect more drastically the maze-learning ability in females. However, these results do not demonstrate an interference of the perinatal DZ insults on the sexual differentiation...
response inhibition, the perinatal DZ effects on performance are more apparent in complex discrimination situations.

It also has been found that these teratogenic effects of DZ on complex maze learning are closely related to the differential DZ-vulnerability of male and female rats at different periods of development: whereas perinatally administered DZ impairs complex maze learning in males but not in females (5, 9), postnatal DZ seems to affect more drastically the maze-learning ability in females (5). On the other hand, these differential effects of DZ depend upon the type of behavior, since in the open field only female activity was affected by DZ administration at a high dose (20 mg/kg) in the last week of pregnancy (9). These results could be interpreted as an interference of DZ with the sexual differentiation process which takes place during the perinatal period. This interpretation, however, is hampered by the lack of evidence of sex differences in the control animals in the later study (9).

It is well known that male rats present higher rates of response than females under continuous reinforcement (CRF) schedule in the Skinner box (4, 11, 23–25), and recently it has been found that this sex difference is controlled by testosterone levels early after birth (4, 25). Moreover, CRF situation implies a simple learning which does not involve a response inhibition process, therefore, this schedule is appropriate to study: (a) the likely effect of DZ on the sexual differentiation process and (b) the effects of perinatal DZ administration on a simple learning situation not requiring a response inhibition. Consequently, in the present work we studied the effects of prenatal (last week of pregnancy) and/or postnatal (from the day of birth to day 16) DZ administration on male and female behavior under CRF schedule in the Skinner box. Additionally, in this study we include an evaluation of the effects of these DZ treatments on activity using the same animals exposed to the open field at the age of 60 days, in order to investigate at this age the effects of perinatal DZ administration on this behavior, and because the sex differences observed in learning situations have been frequently interpreted on the basis of the greater activity levels displayed by female rats (2).

METHOD

Subjects

Fifty-one male and 43 female rats of the Wistar strain (Sepal, Madrid, Spain) were randomly divided into the following groups: (a) 12 males and 7 females, daily exposed, prenatally and postnatally to 2.5 mg/kg of DZ (Groups PP); (b) 11 males and 10 females prenatally exposed to the DZ vehicle (40% propylene glycol in 0.9% saline) and daily injected postnatally with the same dose of DZ (Groups Post); (c) 12 males and 10 females prenatally exposed to DZ vehicle and postnatally injected with the same dose of DZ (Groups Pre); (d) 8 males and 8 females receiving DZ vehicle in both prenatal and postnatal periods (Groups Veh), and (e) 8 males and 8 females nontreated (Groups Nt). Prenatal treatments were carried out through daily subcutaneous (SC) injections to a dam during the last week of pregnancy and postnatal treatments were implemented through daily SC injections to the pups from the day of birth to day 16. After birth all pups (with the exception of the nontreated rats) were nursed by vehicle-injected dams throughout the last week of gestation. During the experiment, animals were located in a room maintained at 18 ± 2°C with a controlled light-dark cycle (light: 07.00 to 19.00). Food and water were freely available.

Apparatus

Activity. One circular Delbarre open field, 90 cm of diameter and 42 cm high, with automatic control through photocells and photoeams located each one 2.5 cm above the floor, Central light and aversive noise were deactivated. The apparatus was divided into six squares controlled by photocells and photoeams.

Learning. Two similar Gerbrands conditioning chambers (model G-7445) enclosed in a sound-attenuated cubicle were used. Each chamber was illuminated throughout the experimental sessions with a white bulb located on the right corner of the transparent ceiling. Light intensity was 10 lux. White noise of 41 dB was constantly delivered through a speaker mounted behind the front wall of the chamber, an additional masking noise was provided by the sound of the ventilating fan.

Each chamber contained two retractile levers located 7.5 cm from the chamber floor, two reward magazines located 3 cm under each lever and two lever lights of 40 mA located 6 cm above each lever. In addition, the reward magazine was illuminated by a 40 mA bulb. In the present experiments, only the right lever and the right food magazine were used. Apparatus and data recording were controlled with two Commodore 64 microcomputers.

Procedure

Activity. At the age of 60 days, animals previously caged in groups of three or four subjects of the same sex and treatment since the age of 21 days were tested in the open field during five consecutive days in a 2-minute daily session. At the beginning of each session, animals were placed in the center of the apparatus and the number of squares crossed were recorded as a general activity measure. Apparatus was cleaned after every subject.

Learning. At the age of 80 days, subjects were individually caged and, prior to the experimental sessions, they were handled (5 min/day/subject) and reduced to 80% ± 10 g of their saturated body weights during ten days, the level at which they were maintained through the whole experiment. On day 1 of the pretraining period subjects were adapted to the operant chamber during 15 minutes with the house light on, feeder light on, the lever retracted, and 40 × 45 mg Noyes pellets available in the lighted feeder. On day 2 feeder training was carried out in a single session of variable-time. During this session subjects received 2 Noyes pellets on an average of 60 seconds during 15 minutes with the lever retracted and the house and feeder lights on. Beginning on the day following feeder training, all rats were trained to press the lever on one day in a CRF free-operant session with the house and the feeder light on. This CRF session lasted 30 responses and each response was reinforced with two Noyes pellets. The experimental training, which lasted four consecutive days, consisted in a daily session of 60 free-operant CRF responses in which each one was reinforced with one pellet.

Statistical treatment of data. Data (number of squares crossed in the open field and number of responses per minute in the Skinner box) were submitted to a two independent three-way analyses of variance involving one factor of repeated measures (days). The analysis of learning was performed without one PP-female because this subject did not reach the learning criteria in the last session of the pretraining period. Post hoc comparisons between groups were implemented using the Tukey's test.

RESULTS

Activity

Male rats presented fewer squares crossed than females [males: 48.1; females: 64.3; Sex: F(1,84) = 27.02, p < 0.0001]. In addition, the analysis of variance also showed a significant main effect of Treatment, F(4,84) = 8.26, p < 0.0001. However, Sex did not interact with the Treatment variables in a significant manner, F(4,84) = 1.06, p > 0.05. As can be seen in Fig. 1, although there
process because in these studies there was no evidence of sex differences in the control animals (5,9). By contrast, in the present work we have obtained clear sex differences in a CRF situation. These results are in agreement with those previously reported by us (4) and by other authors (11, 23-25). Furthermore, it is well known that this sex difference is controlled by the testosterone levels early after birth. Female androgenization and male castration in the early postnatal period reversed in adulthood the direction of sex differences found between male and female control rats (4,25). This period coincides with that in which DZ administration was effective in altering CRF sexual dimorphism by decreasing the male rate. Thus, this DZ effect could be interpreted as a true interference with the CRF sexual differentiation process that normally occurs during the early postnatal life. However, DZ did not interfere with the same process in the open field situation which also has been found to take place in the same period (19). It has been demonstrated that sex differences in the open field depend upon the organizational influences of ovarian hormones (19). Conversely, we recently found (unpublished) that sexual differentiation of the CRF performance is controlled by androgen influences (dihydrotestosterone) in the early postnatal period. So the aforementioned discrepancy could be explained as an interference of DZ with the normal sexual differentiation process when this process depends on the presence of androgens during early postnatal life but not when the estrogens are involved.

Benzodiazepines enhance GABA activity on chloride ionophore (21) and the maximal concentration of the rat brain DZ receptors is reached (3) during the same period in which we have been able to alter CRF sexual dimorphism by postnatal DZ treatment. This suggests that GABA and benzodiazepines could interact at central level with androgens during the stages of neural development and differentiation processes causing a teratological effect on the CRF sexually differentiated behavior. Beside this possible central mechanism, peripheral mechanisms cannot be disregarded to explain the effect of postnatal DZ exposure on the sexually dimorphic CRF performance, because benzodiazepine receptors were found in several peripheral organs including testis (18).

A particularly relevant conclusion of the present results is that an early postnatal DZ treatment is able to affect the sexual differentiation process in the simplest operant learning. Since the human period of brain sexual differentiation appears to occur also postnatally (20), it is not unlikely that the use of DZ in children or in breast-feeding woman could bring about undesired side effects on the normal behavioral development. In any case, our results point to the need of further research on this issue.

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REFERENCES