ALTERATION OF CONVULSIVE THRESHOLD AND SENSITIVITY TO CNS ACTING DRUGS IN SEDATIVE-HYPNOTICS-EXPERIENCED RAT OFFSPRING

Eijiro TAGASHIRA, Kenzo NAKAO, Tomoko URANO, Tameo HIRAMORI and Saizo YANAURA
Department of Pharmacology, Hoshi College of Pharmacy, Ebara 2-4-41, Shinagawa-ku, Tokyo 142, Japan
Accepted November 27, 1981

Abstract—The present study was made to investigate the developmental toxicity of ethanol and several sedative-hypnotics (barbital, diazepam, phenobarbital) administered to rat pups via dams for 18 days (day 3–21 after parturition) from the relatively early postnatal stage in terms of changes in the threshold for pentetrazol (PTZ)-induced convulsion, tolerance to, and physical dependence on barbital (B). In the pups of all dosed groups, the convulsive threshold for PTZ is reduced significantly compared with that in the naive group. The sleeping time with B in phenobarbital-exposed offspring was also reduced significantly compared with that in the naive pups. No difference was however, noted in the B concentration of the brain or plasma upon awaking between these groups of pups, which proved that the phenobarbital-exposed pups had obviously acquired a functional tolerance to B. B-dependence formation and the ensuing weight loss and appearance of withdrawal signs, especially clonic-tonic convulsion, were made intense in the phenobarbital-experienced pups. From these results, it may be concluded that exposure to sedative-hypnotics from the early CNS developing stage makes a lowered state of convulsive threshold for PTZ, and therefore, tends to enhance B-withdrawal convulsion. These phenomena were discussed from the viewpoint of monoamine metabolism in the brain.

Bleyer and Marshall (1) as well as Desmond et al. (2) reported clinical cases of infantile barbital withdrawal signs such as convulsion and tremors. Ouellette et al. (3) described relations of alcohol ingestion to teratogenesis in the newborns. Also, there are papers on experimental studies of latent toxicity of sedative-hypnotics or ethanol applied to dams in the fetal organogenetic or lactation stage (4–7). Yanai and Tabacoff (8) reported that adult mice exposed to a barbiturate in the fetal stage acquired the tolerance to the drug more rapidly than naive offspring. However, there are few reported data of alteration in convulsive threshold when sedative-hypnotics were given postnatally.

Recently, biochemical aspects of tolerance to and physical dependence on barbiturates were mainly discussed from the viewpoint of metabolism of monoamines and other neurotransmitters in the brain (9). In this experiment, we have focused on the relationship between convulsive threshold for pentetrazol (PTZ) and barbital (B) withdrawal convulsion since typical withdrawal
signs of sedative-hypnotics were known to be withdrawal clonic-tonic convulsions.

From our previous study (10), we reported that when weanling rats were dosed with nitrazepam (NZP), an antianxiety drug, from the relatively early postpartal through the lactation stage, they showed severe withdrawal signs including clonic-tonic convulsions in spite of apparently shorter application of NZP than in adult rats. Furthermore, the NZP-exposed offspring showed significantly low threshold values for PTZ-induced convulsions at 6 to 7 weeks (adult) of age. The present study was made to investigate whether these developmental toxicity effects observed in NZP-experienced offspring was general in other sedative-hypnotic agents or not. In this study, changes in convulsive threshold, degree of convulsion from B withdrawal and sensitivity to CNS acting drugs were observed when ethanol or several kinds of sedative-hypnotics were administered at the early CNS developing stage.

MATERIALS AND METHODS

In all experiments, Sprague-Dawley rats (Tokyo Laboratory Animals Co., Tokyo) were used. These rats were reared in an air-conditioned animal room maintained at 22±2°C and illuminated on a 12-hr cycle (the light going on at 8.00 A.M. and going off at 20.00 P.M.). Each nulliparous female (10 to 12 weeks old, weighing 200 to 220 g) was mated with a male (weighing 400 to 500 g) in a cage overnight, and the following day, on the morning of which the vaginal plug was found on the female, was defined as day 0 of gestation. Each pregnant rat was reared in a metal screen cage through day 19 of gestation, and then transferred into a littering cage with its floor covered with wood chips for littering. The day of littering was defined as day 0 post partum. The litter size was adjusted to 8 to 10 pups, and the dams were allowed to nurse the pups.

General behavior throughout the dosing and withdrawal periods: All pups were observed for changes in body weight throughout the dosing and withdrawal periods, and at the same time, the number of survivors and the survival rate were calculated. Also, all dams and pups were examined for withdrawal signs and changes in body weight during withdrawal.

PTZ-induced convulsion: The pups together with their dams were divided into 6 groups of 4 to 6 litters. The dams were dosed with the drugs mixed into their feed during days 3–21 post partum, with the exception of ethanol which was mixed with the drinking water. The groups were treated as follows: Group I: Untreated group, the dams which were fed on a normal food. Group II: Group, the dams which were dosed with 0.5 mg of phenobarbital (phB) per g of food (average daily intake: 55.3 mg/kg body weight). Group III: Group, the dams which were dosed with 2 mg of barbital (B) per g of food (average daily intake: 258 mg/kg body weight). Group IV: Group, the dams which were dosed with 1 mg of diazepam (DZP) per g of food (average daily intake: 128.8 mg/kg body weight). Group V: Group provided with a pair of water bottles in each cage, one of them containing 5% (v/v) of ethanol and the other containing 10% (v/v) of ethanol (average daily intake: 12.9 g/kg body weight). Group VI: Group provided with water bottles containing only 10% (v/v) of ethanol (average daily intake: 17.8 g/kg body weight).

All these groups were withdrawn from the respective drugs on day 22 post partum, and then given free access to a normal food and tap water until they were 6 to 7 weeks old. At the age of 6 to 7 weeks, the male pups (weighing 133.8±7.5 g) were injected subcutaneously with 80 mg of PTZ per kg of
body weight contained in a solution volume adjusted with physiological saline to 0.2 ml per 100 g of body weight. The time to the appearance of prodromal signs of convulsion ("kangaroo-like posture or twitching of the forelimbs) was defined as onset time. The time to the appearance of clonic-tonic convulsion (CTC) after the injection of PTZ was defined as CTC time. The pups were observed grossly for changes in general behavior for 30 min after the injection of PTZ.

Sleeping time: The pups together with their dams were divided into the following 2 groups of 4 litters, and the dams were left untreated or dosed as described during days 3–21 post partum: Group VII. Untreated group, the dams which were fed on a normal food. Group VIII: Group in which the dams were dosed with 1 mg of phB per g of food (average daily intake: 105 mg/kg body weight).

These 2 groups of pups were injected intraperitoneally with 250 mg/kg of sodium barbital in a solution volume adjusted with physiological saline to 0.2 ml per 100 g of body weight at the age of 6 to 7 weeks. The sleep time and the B concentrations in the brain and blood upon awaking were measured. The sleep time was defined as the time from the disappearance until the recovery of righting reflex. The B concentrations in the brain and blood upon awaking were determined by a modification of Broughton's method (11).

Rotarod performance test: The pups together with their dams were divided into the following 3 groups of 5 litters: Group IX: Untreated group, the dams of which were fed on a normal food. Group X: Group in which the dams were dosed with 1 mg of phB per g of food during days 3–21 post partum. Group XI: Group in which the dams were dosed with 1 mg of phB per g of food from day 7 of gestation through day 21 post partum.

The rotarod performance test (9 cm, 5.3 r.p.m., for 3 min as standards; Natsume Seisakusho Co., Tokyo) was performed on the pups of these 3 groups at the age of 6 to 7 weeks. That is, this test was made at the given hours every day during the dosing of the dams with 3 mg of B per g of food for 18 days and with 4 and 6 mg of B per g of food for the 10 ensuing days, to observe the pups for acquisition of tolerance to the inhibition of motor incoordination with B with the passage of time. Also in order to examine the pups for the formation of physical dependence on B, the dams were treated with graded increases of doses of B from 0.5 and 1 to 6 and 8 mg/g food over 36 days following the method of Tagashira et al. (11); the pups were examined for acquisition of physical dependence on B and withdrawal signs, and the findings were compared with those in naive pups.

RESULTS

General behavior throughout dosing and withdrawal periods: Table 1 shows the number of pup survivors at day 21 post partum, the pup survival rate in the untreated group and the groups dosed during the lactation period, and also the number of pup survivors and the pup mortality rate within 72 hr of drug withdrawal. The number of pup survivors and the pup mortality rate throughout the dosing period indicated that the dosing of the dams with 2 mg of B/g of food or with 5 and 10% ethanol had little influence on the pup survival rate (97.7 and 95.7%) as compared with the naive pup survival rate of 91.3%. On the other hand, treatment of the dams with 0.5 mg of phB/g of food, 1 mg of DZP/g of food, or the high dose of ethanol resulted in significantly lower pup survival rates such as 72 to 78% (P<0.05). Figure 1 illustrates the pup body weight curves during the dam-dosing period.
Table 1. Survival ratio of offspring during administration of sedative-hypnotics via dams fed the drug-admixed food (day 3 to 21 after parturition), and mortalities of offspring after the drug withdrawal

| Treatment with drugs | Survival ratio during drug administration(%) | Mortalities during 72 hr withdrawal(%) |
|----------------------|-----------------------------------------------|---------------------------------------|
| Naive control        | 42/46 (91.3)                                  | 0/42 (0)                              |
| Phenobarbital (0.5 mg/g food) | 42/58 (72.4)                           | 1/42 (2.4)                            |
| Barbital (2 mg/g food) | 42/43 (97.7)                               | 3/42 (7.1)                            |
| Diazepam (1 mg/g food) | 31/40 (77.5)                           | 2/31 (6.5)                            |
| Ethanol (5 and 10%)  | 35/46 (76.1)                               | 0/35 (0)                             |
| Ethanol (10%)        | 44/46 (95.7)                               | 0/44 (0)                             |

All drugs were administered to rat pups via dams for 18 days (day 3 to 21 after parturition) from the relatively early postnatal stage.

Fig. 1. Growth curve of body weight of neonates treated with the drugs after parturition. Each drug was administered to rat pups via dams fed drug-admixed food except for ethanol which was administered in drinking solution for 18 days (day 3-21 after parturition).

Fig. 2. Time course for changes in body weight of dams after withdrawal from the drug. Each dam ingested drug-admixed food, or ethanol in drinking water for 18 days (day 3-21 after parturition). On day 21, drug-admixed food was replaced with normal drug-free food.

The pups of the dams dosed with 5 and 10% of ethanol or 2 mg of B/g of food showed a weight gain curve similar to that of the control pups. However, the pups of the dams treated with 0.5 mg of phB/g of food, 1 mg of DZP/g of food, or 10% of ethanol showed retardations in weight gain. None of the pups showed any particular change in general behavior during the dosing period, but the dams exhibited mild CNS suppression (ptosis and muscle relaxation) only in the early dosing period. A tendency for a somewhat high pup mortality rate was noted in the groups dosed with 0.5 mg of phB/g of food, 2 mg of B/g of food, or 1 mg of DZP/g of food (2 to 7%). Both dams and pups were observed for withdrawal signs and changes in body weight between 24 and 72 hr of withdrawal. The dams treated with either dosage level of ethanol showed no weight loss or withdrawal sign. The dams dosed with phB, B or DZP exhibited 8 to 10%
weight losses (Fig. 2), while they showed no severe withdrawal signs such as convulsions but only mild ones as a whole. The pups showed no weight loss or withdrawal signs (Fig. 3).

**PTZ-induced convulsion:** In the pups of all dosed groups except in those of group V, the onset time and the CTC time were reduced significantly ($P<0.01$) compared with these parameters in the naive group. Also, in the pups of all groups dosed with phB, B and DZP, the incidence of prodromal signs of convulsion and CTC was 100% (8/8, 10/10 and 10/10 pups) compared with the incidence of 90% (9/10 pups) in the naive group: the incidence also tended to be higher in the drug-exposed groups (Fig. 4).

**Sleeping time:** The sleeping time in pups of group VII was reduced significantly ($P<0.01$) compared with that in the naive pups. However, no difference was noted in the B concentration of the brain or plasma upon awaking between these groups of pups, proving that the phB-exposed pups had obviously acquired functional tolerance to B (Table 2).

**Rotarod performance test:** The daily B intake by the pups of group X was mostly equal to that by the pups of group IX or those of group XI. However, the inhibition of motor coordination with B scarcely

---

Fig. 3. Time course for changes in body weight of neonate after withdrawal from the drug. Each drug was administered to neonates via dams fed drug-admixed food except for ethanol which was administered in drinking solution from day 3 to 21 after parturition.

---

Fig. 4. Threshold value for pentetrazol-induced convulsion in drug-experienced offspring (6–7 weeks of age). During the weaning period, each drug was administered to rat pups via dams fed drug-admixed food except for ethanol which was given in drinking solution for 18 days (day 3–21 after parturition).
developed throughout the B dosing period, and the signs of CNS suppression remained mild. Also, in the pups of group XI, the inhibition of motor coordination of pups with B during the dosing of the dams with 3 mg of B/g of food was less severe than that in the pups of group IX. At 4 and 6 mg of B/g of food, however, a tendency for more severe motor coordination inhibition with B was noted in the pups of group XI than in the pups of group IX (Fig. 5).

B dependence formation test: B dependence formation and the ensuing weight loss and appearance of withdrawal signs (hyperirritability, ataxia, tremor, muscle rigidity, clonic-tonic convulsion) were intense in the pups of groups X and XI compared with those in the naive pups of group IX. The pups of group X exhibited severe convulsions in the early stage of withdrawal, one of the 6 pups dying immediately after the convulsions. Furthermore, the pups of groups X and XI showed obviously slow recovery of withdrawal signs. Especially in the pups of group XI, vital signs such as anorexia and weight loss did not recover to their levels before the withdrawal even at 3 weeks of withdrawal (Fig. 6).

| Treatment with drug | Sleep time (min) | Brain (μg/g) | Plasma (μg/ml) |
|---------------------|------------------|--------------|---------------|
| Naive control       | 409.2±17.0       | 30.1±1.4     | 94.1±3.0      |
| Phenobarbital       | 334.8±23.0*      | 31.8±1.8     | 81.3±7.5      |

Phenobarbital was administered to rat pups via dams fed phenobarbital-admixed food (1 mg/g food) for 18 days (day 3 to 21 after parturition). Significantly different from the naive control, *P<0.01.

Fig. 5. Time course of tolerance development to motor incoordination with barbital in phenobarbital-experienced offspring. Phenobarbital (1 mg/g food) was administered to dam-neonates during gestation (day 7-17) and/or weaning period (day 3-21 after parturition). Motor incoordination with barbital (3 mg/g food) was measured by the rotarod performance test.
DISCUSSION

In recent years, attention has been paid to the study of toxicity that appears in neonates when dams are dosed with drugs during the pre- and postnatal periods, even among the reproduction studies of new drugs. This toxicity is generally studied by observing changes in general behavior or from the viewpoint of behavioral pharmacology (12), although there are papers on studies of latent toxicity of CNS-acting drugs in drug sensitivity (13). Friedler and Cochin (14) reported that the offspring from the dams exposed to morphine before gestation showed retarded growth compared with those from naive dams, suggesting the probable occurrence of genetic abnormality.

In the present study, the dosing of dams with sedative-hypnotics throughout the 21-day lactation period resulted in no change in either body weight or daily food consumption of dams compared with either parameter in untreated ones. However, it was associated with the deaths of pups from the dosed dams in the early stage of dosing due presumably to inhibited nursing ability. Because such doses of the drugs as to inhibit the CNS of dams moderately (causing ptosis, systemic muscle relaxation, staggering gait) were used in this study, the pups were sometimes scattered in the nursing cages, and about 20% of them seemed to have died from failure to ingest sufficient milk (except for those of groups III and V). A tendency for retardation in growth of pup survivors (weight gain) was also noted in these groups. Because about 70 to 80% of pups available at the start of dosing survived as a whole, these may be said to be optimal maximal tolerable doses for use in a perinatal experiment.

During withdrawal of each drug, the dosed dams except for those given ethanol showed significant weight losses, while their pups exhibited virtually no withdrawal signs. This finding suggested that the test drugs might give obviously different actions on rat pups compared with the action of nitrazepam described in a previous paper (9) that the drug caused severe withdrawal signs (including convulsions) in developing pups. Since NZP induced signs similar to those of
enhancement of the serotonergic system such as head shaking, "wet dog" shaking (caused with e.g., tryptophan, 5-hydroxytryptophan), actions of NZP on serotonin (5-HT) metabolism in the brain of developing pups seem to specifically differ from the drugs used in the present experiment.

However, the treatment of rat pups with phB, B, DZP or ethanol via dams during the lactation period lowered the convulsive threshold for PTZ as in the study with NZP. Yanai et al. (15) observed that the exposure to ethanol of C57BL/6J mouse neonates via dams from the fetal stage through day 14 post partum elevated the sensitivity to audiogenic seizure, and they suggested that because this seizure was inhibited with monosodium glutamate and 5-hydroxytryptophan (5-HTP) but intensified with p-chlorophenylalanine (PCPA), the ethanol-exposed mice probably had some functional abnormality in the serotonergic system. On the other hand, the threshold value for PTZ-induced convulsion in naive rats is lowered with PCPA pretreatment or propranolol, a β-adrenolytic agent, but somewhat elevated with 5-HTP pretreatment (16). Bhattacharya and Sanyal (17) also reported relations of PTZ-induced convulsion to the CNS monoamines.

Our laboratory (18, 19) found that the 5-HT synthesis rate was elevated in adult rats during B withdrawal. We further showed that not only 5-HT related compounds but also α-methyl-p-tyrosine and β-adrenoceptor blockers inhibited B withdrawal convulsion, indicating that the mutual balance between the noradrenergic and the serotonergic neurons might play an important role in the development of B withdrawal convulsion.

These results suggest that the reduced convulsive threshold for PTZ in ethanol- and sedative-hypnotics-exposed rats noted in the present study might probably have been derived from a metabolic abnormality in the CNS monoaminergic neurons or an imbalance of neural activities due to the dosing during the CNS developing period.

When the rat pups exposed to phB during the lactation period were compared with those exposed to the drug throughout the fetal and lactation periods, the former rather showed a tendency for a reduction in rotarod performance inhibition. One of the causes for this reduction seems to be the reduction in the effect of postnatal phB due to the phB induction (metabolic tolerance) during the fetal stage onward (20). Challenge with phB from immediately after birth onward may therefore be thought to be more influential on the CNS monoaminergic neurons involved in B tolerance development (21).

The development of tolerance to B in the phB-exposed rats with the passage of time was markedly lessened as a whole, instead of persistent hyposensitivity to B of the CNS. Furthermore, the formation of physical dependence on B and the ensuing withdrawal signs, especially clonic-tonic convulsions, were intensified and some of them died of the convulsion. The relation of the aforementioned hyposensitive reaction to B to dependence on B suggests that the mechanism of tolerance development may differ from that of dependence formation and also from that for elicitation of withdrawal convulsion.

In conclusion, these sedative-hypnotics-experienced pups might have already been in a condition where convulsions were likely to occur.

REFERENCES

1) Bleyer, W.A. and Marshall, R.E.: Barbiturate withdrawal syndrome in a passively addicted infant. J. Am. med. Ass. 221, 185–186 (1972)
2) Desmond, M.M., Schwanecke, R.P., Wilson, G.S., Yasunaga, S. and Burgdorff, I.: Maternal barbiturate utilization and neonatal withdrawal symptomatology. Pediatrics. Springfield 80, 190–197 (1972)
3) Ouellette, E.M., Rosett, H.L., Rosman, N.P.
and Weiner, L.: Adverse effects on offspring of maternal alcohol abuse during pregnancy. New Eng. J. Med. 8, 528–530 (1977)

4) Bond, N.W. and Di Giusto, E.L.: Effects of prenatal alcohol consumption on openfield behavior and alcohol preference in rats. Psychopharmacol. 46, 163–165 (1976)

5) Yanai, J. and Ginsburg, B.E.: Long term reduction of male agonistic behavior in mice following early exposure to ethanol. Psychopharmacol. 52, 31–34 (1977)

6) Murai, N.: Effect of maternal medication during pregnancy upon behavioral development of offspring. Tohoku J. exp. Med. 89, 265–272 (1966)

7) Schain, R.J. and Watanabe, K.: Origin of brain growth retardation in young rats treated with phenobarbital. Expl. Neurol. 50, 806–809 (1976)

8) Yanai, J. and Tabacoff, B.: Increased tolerance in mice following prenatal exposure to a barbiturate. Psychopharmacol. 64, 325–327 (1979)

9) Ho, I.K.: Mechanism of action of barbiturates. Annu. Rev. Pharmacol. Toxicol. 21, 83–111 (1981)

10) Tagashira, E., Urano, T., Nakao, K., Hiramori, T. and Yanaura, S.: Secondary withdrawal signs in nitrazepam-experienced rat offspring. Abstract of the 6th Annual Meeting of the Japanese Society of Toxicological Sciences, p. 60 (1981) (in Japanese)

11) Tagashira, E., Izumi, T. and Yanaura, S.: Experimental barbiturate dependence 1. Barbiturate dependence development in rats by Drug-Admixed Food (DAF) method. Psychopharmacol. 57, 137–144 (1978)

12) Krsiak, M., Elies, J., Poschlova, N. and Masek, K.: Increased aggressiveness and lower brain serotonin levels in offspring of mice given alcohol during gestation. J. Stud. Alcohol 38, 1666–1704 (1977)

13) Rawat, A.K.: Developmental changes in the brain levels of neurotransmitters as influenced by maternal ethanol consumption in the rat. J. Neurochem. 28, 1175–1182 (1977)

14) Friedler, G. and Cochin, J.: Growth retardation in offspring of female rats treated with morphine prior to conception. Science 175, 654–656 (1972)

15) Yanai, J., Sze, P.Y. and Ginsburg, B.E.: Effects of aminergic drugs and glutamic acid on audiogenic seizures induced by early exposure to ethanol. Epilepsia 16, 67–71 (1975)

16) Kilian, M. and Frey, H.H.: Central monoamines and convulsive thresholds in mice and rats. Neuropsychopharmacol. 12, 681–692 (1973)

17) Bhattacharya, S.K. and Sanyal, A.K.: Inhibition of pentylenetetrazol-induced convulsions in rats by prostaglandin E1: Role of brain monoamines. Psychopharmacol. 56, 235–237 (1978)

18) Tagashira, E., Urano, T. and Yanaura, S.: The role of brain serotonin in dependence on barbiturates and subsequent withdrawal convulsions. Japan. J. Pharmacol. 30, Supp. 198P (1980)

19) Tagashira, E., Urano, T., Nakao, K., Hiramori, T. and Yanaura, S.: Inhibitory effects of propranolol on barbital-withdrawal convulsion precipitated with monoamine oxidase inhibitor. Folia pharmacol. jap. 78, 57P–58P (1981) (in Japanese)

20) Kato, R.: The metabolism of drugs in the pregnant, fetus and newborn rats, and the effect of the administration of drugs on their activities. Folia pharmacol. jap. 61, 148P (1965) (in Japanese)

21) Tabakoff, B., Yanai, J. and Ritzman, R.F.: Brain noradrenergic systems as a prerequisite for developing tolerance to barbiturates. Science 200, 449–451 (1978)