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BRIEF COMMUNICATION

Avoidance Behavior, Prolactin, HVA and DOPAC in Offspring of Bromopride-Treated Rats

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NASELLO, A. G. AND L. F. FELICIO. *Avoidance behavior, prolactin, HVA and DOPAC in offspring of bromopride-treated rats.* PHARMACOL BIOCHEM BEHAV **37**(3) 571–575, 1990.—The effects of perinatal treatments with bromopride (BRO), a dopaminergic blocking agent, on serum prolactin (PRL), striatal dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels, and active and inhibitory avoidance behavior of both sexes, were examined in adult Wistar rats. Offspring were divided into four groups depending upon the treatment received by the dams: BV—offspring of dams exposed to BRO only during pregnancy; VB—offspring of dams receiving BRO only during lactation; BB—offspring of dams treated with BRO during pregnancy and lactation; and VV—offspring of dams receiving vehicle during both periods. Active avoidance responses were reduced in males of all experimental groups. Other parameters such as inhibitory avoidance, serum PRL levels, and striatal HVA and DOPAC levels were not different from the control group. The results suggest that there is no relationship between HVA and DOPAC striatal levels, serum PRL levels and the behavioral modifications described here.

Bromopride Perinatal treatment Avoidance behavior Prolactin HVA DOPAC

PERINATAL treatment with dopaminergic blocking agents leads to biochemical, behavioral and/or hormonal changes in adult rat offspring (3–5, 13, 16). Learning deficits have been described in rats after perinatal treatment with some dopaminergic blocking agents such as haloperidol (2), clozapine (4) and trifluoroperazine (13,14). Striatal metabolism of dopamine, as indicated by striatal levels of dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), decreases after postnatal treatment with haloperidol (2,3) and increases after treatment with clozapine in the same period (4).

Bromopride [N-(dietylaminoethyl)-2-methoxy-4-amino-5-bromobenzamide; BRO], a substituted benzamide (12,23), blocks dopamine D2 receptors (17). BRO is commonly used during pregnancy for its antiemetic properties (1,15) and may be given during lactation since it enhances prolactin levels (23) thereby increasing milk production. It has been shown that different BRO treatments during pregnancy and/or lactation may modify different behavioral parameters in offspring such as motor activity, apomorphine-induced stereotyped behavior (9) and sexual behavior of both sexes (10). Just after weaning, offspring of dams treated with BRO show an increase in general activity and in apomorphine-induced stereotypy, and adult females exhibit lower lordosis quotients.

The present study was undertaken to verify the effects of BRO treatment during pregnancy and/or lactation in learning-related behaviors such as active and inhibitory avoidance. In addition, striatal dopamine metabolism was assessed by measuring DOPAC and HVA levels in order to investigate the possible relationship between the behavioral effects of perinatal BRO treatment and dopamine metabolism. Serum prolactin levels were also measured as an indirect indicator of dopamine metabolism at the tubero-infundibular level (29).

METHOD

Animals

Adult albino rats of Wistar origin were used. They weighed 200–300 g and were housed at a controlled temperature (22–23°C) under 12-hr light/dark cycle (lights off at 0600 p.m.). Food and water were available ad lib throughout the experiments. Forty female rats with conditioned avoidance response of 10 to 90% (see below) were selected and mated with male rats selected with the same criterion. Female rats were treated with BRO (30.0 mg/kg) or with its vehicle subcutaneously from the first day of pregnancy until delivery and/or weaning on day 21 of postnatal life. Bro-

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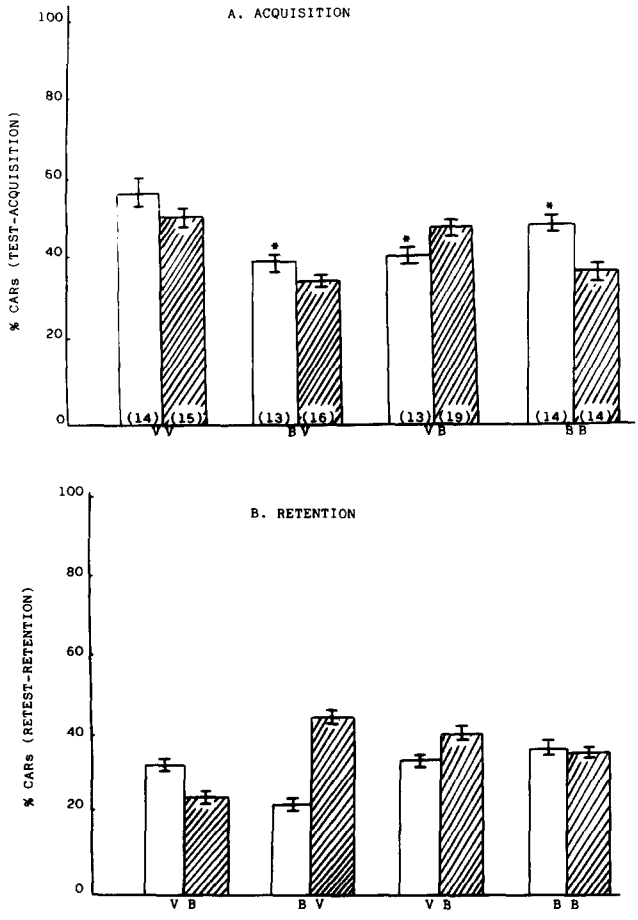

FIG. 1. Influence of perinatal treatments with bromopride on acquisition (A) and retention (B) of CARs of adult male (open bars) and female (hatched bars) rats. The groups were formed from offspring of dams treated with vehicle only (VV), bromopride during pregnancy only (BV), throughout pregnancy and lactation (BB) and during lactation only (VB). Bars represent mean values and the vertical lines the SE. Number of animals per group are in parentheses. *$p<0.05$ compared to the corresponding VV group (Kruskall-Wallis/Mann-Whitney U two-tailed).

mopride (Roche) was dissolved in 0.2 M monopotassium phosphate solution (pH adjusted to 5.0 with hydrochloric acid). The drug was administered subcutaneously in a volume of 1.0 ml/kg of body weight. Offspring were divided into four groups depending upon the treatment received by the dams: BV—offspring of dams exposed to BRO only during pregnancy; VB—offspring of dams receiving BRO only during lactation; BB—offspring of dams treated with BRO during pregnancy and lactation; and VV—offspring of dams receiving vehicle during both periods. Onset of pregnancy was determined by the presence of spermatozoa in vaginal smears taken daily. Offspring of similar age were distributed among mothers within the same experimental group, in such a way that each mother had 4 male and 4 female pups. The litters were examined at birth for possible teratogenic effects and weighed daily. Litters which deviated markedly at birth from a 1:1 sex ratio, or from a litter size of between 8 and 14 were not assigned to the experiment. Male and female animals were tested for active and inhibitory avoidance at 90 days of age. The tests were performed between 1000 and 1500 hr.

TABLE 1

NONMOTIVATED RESPONSES (MEANS ± SE) OF ADULT MALE AND FEMALE RATS FROM DAMS THAT WERE TREATED WITH BROMOPRIDE DURING THE PERINATAL PERIOD

	Group*			
	VV	BV	VB	BB
Males	11 ± 1.7 (14)†	7 ± 0.9‡ (13)	9 ± 1.3 (13)	6 ± 0.5‡ (14)
Females	22 ± 3.2 (15)	11 ± 2.0‡ (16)	16 ± 3.0 (19)	13 ± 1.0‡ (14)

*Rats of mothers treated with the bromopride vehicle during the pregnancy and lactation (VV); mothers injected with bromopride during pregnancy and vehicle during lactation (BV); mothers treated with vehicle during pregnancy and bromopride during lactation (VB); mothers receiving bromoprode during pregnancy and lactation (BB).

†Number of animals.

‡$p<0.05$ compared to corresponding VV group (Kruskal-Wallis/Mann-Whitney U two-tailed).

Active Avoidance Task

Animals were tested in a two-way active avoidance task. The apparatus was a 50 × 25 × 25 cm automated acrylic box (Albarsch) with a floor that consisted of a series of 1 mm caliber parallel bars divided at midline by a 1 cm high acrylic hurdle. The conditioned stimulus was a 5 sec, 5 dB, 0.75 KHz tone delivered through a loudspeaker attached to the rear wall of the box. Each tone was immediately followed by a 0.5 mA footshock (unconditioned stimulus). Animals were allowed to explore the box for 5 minutes and then received 50 tone footshock trials with an intertrial interval of 30 sec. Animals avoided the shocks by crossing the hurdle during the tone [conditioned avoidance response (CAR)]. Each animal received two sessions of the shuttle avoidance condition with an interval of seven days between them. In the first or acquisition session, the animal was trained over 50 trials, in the second or retest session, the rat was retrained over the same number of trials. "Retention" was considered as the difference in the same animal's performance between the two sessions. The latencies for crossing were also measured in every case. When an animal crossed without any stimuli it was considered a nonmotivated response (NMR).

Inhibitory Avoidance Task

The inhibitory avoidance conditioning was carried out using an adaptation of the method described by Denti and Epstein (6). The apparatus employed was a two-way shuttle-box provided with a manual guillotine door placed between the two modular testing chambers. One chamber was illuminated by a 40-watt light, while the other remained dark. Foot shocks were applied through the grid floor in the dark chamber. Each animal received one trial on each of two consecutive days. In each trial the rat was placed in the illuminated chamber facing away from the guillotine door. When the rat entered the darkened chamber the door was closed quietly and the animal remained there for 10 sec. The latency to enter was recorded. On day 3, when the animal entered the darkened chamber, a 0.8 mA foot shock was applied for 2 sec through the grid floor. The latency to enter was again recorded. The retention test, given on day 4, consisted of a single trial in which

TABLE 2

LATENCIES (MEANS ± SE) TO CROSS AND PERCENTAGES OF INHIBITORY-AVOIDANCE RESPONSES (IARs) OF ADULT RATS FROM DAMS TREATED WITH BROMOPRIDE DURING THE PERINATAL PERIOD

Group*		N	Latency Scores† Day 1	Day 2	Day 3	Day 4	% of IARs
Males	VV	9	20 ± 18.8	7 ± 2.1	9 ± 5.4	300 ± 0.0	100
	BV	14	21 ± 7.7	9 ± 5.4	10 ± 6.7	300 ± 0.0	100
	VB	11	23 ± 9.6	11 ± 4.7	8 ± 4.3	300 ± 0.0	100
	BB	13	23 ± 12.1	12 ± 16.2	7 ± 3.6	300 ± 0.0	100
Females	VV	12	17 ± 11.0	10 ± 6.2	10 ± 13.3	224 ± 33.9	58.3
	BV	12	17 ± 6.4	7 ± 5.2	11 ± 18.5	238 ± 24.5	66.7
	VB	12	13 ± 4.9	7 ± 5.8	7 ± 4.1	171 ± 33.7	58.3
	BB	12	17 ± 15.5	19 ± 24.6	12 ± 15	223 ± 30.6	41.7

*Rats of mothers treated with the bromopride vehicle during pregnancy and lactation (VV); mothers injected with bromopride during pregnancy and vehicle during lactation (BV); mothers treated with vehicle during pregnancy and bromopride during lactation (VB); mothers receiving bromopride during pregnancy and lactation (BB).

†Each rat received one trial a day. In each trial the rat was placed in the illuminated chamber and the latency to entering the dark chamber was recorded. On day 3, after entering the dark chamber, the animals received a foot shock. The retention test was given on day 4.

the animal was again placed in the illuminated chamber and the latency to cross over to the dark chamber was measured, up to a maximum of 300 sec. The latency of 300 sec was considered an inhibitory avoidance response (IAR).

Prolactin Assay

Animals were removed quietly and individually from the cage and transferred to a separate room for decapitation. Trunk blood was collected in tubes, centrifuged and serum was frozen. All animals were sacrificed from 1300 to 1500 hr to avoid circadian variations of serum PRL levels (7,18). To minimize the influence of the estrous cycle on PRL levels, females were always sacrificed in proestrus. PRL levels were measured by radioimmunoassay using materials supplied by the National Pituitary Agency, NIADDK, Bethesda, MD. The concentrations were measured as ng/ml, based on RP-3 as standard.

TABLE 3

CONCENTRATION OF SERUM PROLACTIN (ng/ml) IN ADULT RATS PERINATALLY TREATED WITH BROMOPRIDE

	Groups* VV	BV	VB	BB
Males	16 ± 6† (8)‡	11 ± 3 (14)	21 ± 7 (13)	14 ± 6 (10)
Females	48 ± 18.7 (7)	40 ± 10.3 (11)	38 ± 11.0 (13)	55 ± 13.0 (11)

*Rats of mothers treated with the bromopride vehicle during pregnancy and lactation (VV); mothers injected with bromopride during pregnancy and vehicle during lactation (BV); mothers treated with vehicle during pregnancy and bromopride during lactation (VB); mothers receiving bromopride during pregnancy and lactation (BB).

†Means (±SE).

‡Number of animals.

HVA and DOPAC Assay

The brains were obtained from rats after decapitation, as described above. The striatum was immediately removed and homogenized in 0.1 N perchloric acid. HVA and DOPAC were assayed spectrofluorimetrically after absorption and elution from Sephadex G-10 according to the procedure described by Westerink and Korf (30–32).

Statistical Analysis

For the biochemical measures, an analysis of variance followed by the Duncan's test was used to detect possible differences among groups (27,28). On experiments involving active and inhibitory avoidance, the results were analysed by the Kruskal-Wallis analysis of variance for nonparametric data followed by the Mann-Whitney U-test (26). A probability of $p<0.05$ was considered significantly different for all comparisions made.

RESULTS

There were no differences in the body weight, or the age of eye or vaginal openings among the four groups. Active avoidance responses were reduced in males of all experimental groups compared to control group (Fig. 1A), while female performance was not significantly affected in any experimental group compared to the control group (Fig. 1A). There were no significant differences between groups in the retention of the active avoidance task (Fig. 1B). Nonmotivated responses (NMRs) of male and female rats were reduced in both sexes in groups BV and BB (Table 1). No differences were observed in the crossing latencies (data not shown). In the inhibitory avoidance test there were also no differences among groups either in latencies to cross or in percentages of animals showing IARs on day 4 (Table 2).

There were no differences in mean serum PRL levels among the various groups (Table 3). The levels of HVA and DOPAC were not different among groups (Table 4).

TABLE 4

STRIATAL HVA AND DOPAC CONCENTRATIONS (ng/g) IN ADULT RATS FROM DAMS TREATED WITH BROMOPRIDE DURING THE PERINATAL PERIOD

		Group*			
		VV	BV	VB	BB
Males	HVA	950 ± 65.1† (7)‡	801 ± 45.5 (7)	931 ± 43.1 (8)	928 ± 38.9 (8)
	DOPAC	1133 ± 207.4 (7)	1395 ± 94.1 (7)	1494 ± 80.1 (7)	1535 ± 128.2 (8)
Females	HVA	1016 ± 58.9 (8)	1140 ± 73.3 (8)	1133 ± 73.3 (8)	1087 ± 75.9 (8)
	DOPAC	1515 ± 111.2 (8)	1754 ± 150.4 (8)	1719 ± 88.8 (8)	1667 ± 90.3 (8)

*Rats of mothers treated with the bromopride vehicle during pregnancy and lactation (VV); mothers injected with bromopride during pregnancy and vehicle during lactation (BV); mothers treated with vehicle during pregnancy and bromopride during lactation (VB); mothers receiving bromopride during pregnancy and lactation (BB).

†Means (±SE).

‡Number of animals.

DISCUSSION

The present results indicate that active avoidance behavior is reduced in male rats perinatally exposed to BRO. Inhibitory avoidance behavior was not affected in either male or female rats. Exposure of immature rats to various dopaminergic blocking agents can lead to long-term behavioral changes (5, 16, 20) such as deficits in learning (5,16) and sexual behavior (10).

Perinatal BRO treatment induces behavioral changes in adult rat offspring that are different from those observed in adult animals after abrupt withdrawal of long-term BRO treatment (8) and similar to those observed in acute BRO treatment (11,22). Perinatal and acute BRO treatments induce deficits in locomotion frequency in the adult rat (9,22), on the other hand, withdrawal of long-term BRO treatment induces hyperactivity (8). The results shown here and in previous papers (9,10) confirm this (i.e., behavioral changes observed in adult offspring of mothers treated with BRO are similar to those showed by rats treated acutely with this drug). This similarity between acute and perinatal effects is also observed for dopaminergic stimulant drugs such as amphetamine (19–21, 24, 25) and for dopaminergic blockers such as chlorpromazine, haloperidol, penphluridol and clozapine (2–5, 13, 14, 16).

In the present experiment the drug BRO, a substituted benzamide, was used. The results obtained show that the learning deficits observed in offspring of BRO-treated mothers such as the poor performance in the active avoidance task, are similar to the deficits found in the literature concerning perinatal treatment with neuroleptics (5).

The poor performance in an active avoidance task clearly shown by the experimental male groups may be due to a learning deficit and/or a drug-induced decrease in locomotor activity. These two hypotheses are not exclusive. The lower number of NMRs showed by the BB and BV groups may be due to a lower locomotion frequency (9), however, 1) females of the BB and BV groups, although presenting lower number of NMRs, do not show significant differences from the control group in total percentage of conditioned avoidance responses (%CARs); and 2) the male VB group showed less %CARs than the controls without presenting any significant differences in NMRs. These data suggest that the lower NMRs, a possible consequence of a decrease in locomotor activity, is not the only factor inducing the deficits in the performance of animals of the experimental groups during the active avoidance test.

There were no differences between groups in the inhibitory avoidance test. These data suggest that perinatal BRO treatment does not affect neural mechanisms related to this kind of behavioral response.

Dopamine D2 blockers, such as BRO, stimulate prolactin secretion (11,23) and may produce long-term hyperprolactinemia in the adult offspring of mothers treated with BRO. No differences were found among the various groups, suggesting that the behavioral modifications observed in these animals were not related to altered prolactin levels.

It is known that acute treatment with dopaminergic blocking agents can enhance dopamine metabolism in the striatum (3, 4, 17). It has been demonstrated that the intensity of this response may be modified by perinatal treatment with haloperidol (3) which is a cataleptogenic neuronal drug and with clozapine which is a noncataleptogenic drug (4). Although no differences were observed in HVA/DOPAC striatal levels of adult offspring, the present data do not rule out the possibility that perinatal BRO treatment induced changes in dopamine metabolism. In fact, striatal dopamine metabolites were measured without a previous challange, such as an acute injection of BRO, nor was turnover measured.

Active avoidance in males was modified in all experimental groups. The biochemical measures described here suggest that there is no relationship between striatal dopamine metabolism, serum PRL levels and the behavioral modifications described here and elsewhere (9,10). Further studies are necessary to elucidate the mechanism of the behavioral changes induced by perinatal administration of these kind of drugs.

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