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Effects of Bromocriptine and Haloperidol on Ethanol Withdrawal Syndrome in Rats

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UZBAY, İ. T., E. S. AKARSU AND S. O. KAYAALP. *Effects of bromocriptine and haloperidol on ethanol withdrawal syndrome in rats.* PHARMACOL BIOCHEM BEHAV **49**(4) 969-974, 1994.—The effects of bromocriptine and haloperidol, either alone or in combination, on ethanol withdrawal syndrome (EWS) have been investigated in rats. Bromocriptine (5 mg/kg IP) inhibited wet dog shakes behavior and catatonia but potentiated the intensity of abnormal gait. The latency of the audiogenic seizures was prolonged by bromocriptine treatment. Haloperidol (0.5 mg/kg SC) decreased the intensity of stereotyped behavior but potentiated catatonia and agitation. It did not antagonize the behaviors induced by bromocriptine when injected in combination except the increased latency of the audiogenic seizures. The total intensity score of the EWS was not significantly different from that in untreated control. The results suggest that brain dopaminergic system may be involved to a limited extent in mediating the EWS in rats.

Ethanol withdrawal syndrome Dopamine Bromocriptine Haloperidol Rat

ETHANOL withdrawal syndrome (EWS) precipitated by discontinuing chronic ethanol intake is the most important evidence indicating the presence of physical ethanol dependence (6). Although the signs of EWS in human (19) and rats (9) have been well described, the mechanisms underlying physical dependence or EWS are poorly understood.

Alterations in brain dopaminergic system have been suggested to be involved in ethanol-induced dependence and withdrawal (1,8,13,14). It has been observed that bromocriptine, a dopamine D_2 receptor agonist, attenuated craving for ethanol in alcoholics (3) and reduced ethanol consumption in ethanol-preferring rats in a dose-dependent manner (22). Furthermore, this drug has been used to treat ethanol withdrawal to a limited extent (2,15). Many reports indicate that EWS is associated with decreased activity in dopaminergic neurons and receptors, especially in the striatum (4,5,16-18).

In the present study, the possible role of brain dopaminergic activity on EWS has been investigated in rats by using bromocriptine, a dopamine D_2 agonist and haloperidol, a non-selective dopamine receptor antagonist.

METHOD

Animals and Laboratory

Adult male Wistar rats, 216-235 g weight at the beginning of the experiments were used. They were kept in a quiet and temperature- and humidity-controlled room (22 ± 3°C and 60 ± 5%, respectively) in which a 12 L : 12 D cycle was maintained (0800-2000 h light).

Oral Ethanol Self-Administration

For chronic ethanol exposure, the animals were housed individually and ethanol was presented in the liquid diet according to Parale and Kulkarni (12) with the modification that vitamin A 5000 IU/l was added and sucrose was replaced by cyclamate sodium 93.75 mg/l (Bilim İlaç San., Turkey) and saccharin sodium 9.375 mg/l (Bilim İlaç San., Turkey) (21). The mixture with ethanol supplies 925 kcal/l. At the beginning of the study, rats were given liquid diet without ethanol for 7 days. Then liquid diet with 2.4% ethanol was administered for 3 days. The ethanol concentration was increased to 4.8% for the following 3 days and finally to 7.2% for 20 days. Liquid diet was freshly prepared daily and presented at the same time of the day (1000 h). Ethanol consumption was recorded daily and expressed as g/kg/day.

Drugs

Bromocriptine mesylate (Sandoz, Turkey) and haloperidol (Ali Raif İlaç San., Turkey) were dissolved in 0.5% tartaric acid and injected intraperitoneally (IP) and subcutaneously (SC), respectively, at volumes of 1 ml/100 g.

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Procedure

At the 21st day of exposure to 7.2% ethanol-containing liquid diet, ethanol was withdrawn from the diet by replacing the diet with that without ethanol at 1000 h and the ethanol-dependent rats were divided into seven groups.

Blood ethanol levels were determined in the first three group (n = 5 for each) using a fluoroscence polarization immunoassay method (7) (TDX autoanalyser, Abbott, USA) before removing ethanol from the liquid diet and 6th and 24th hour following the withdrawal. The blood samples were taken by intracardiac puncture in the awake rats.

Bromocriptine mesylate (5 mg/kg IP) and haloperidol (0.5 mg/kg SC) were administered after discontinuing ethanol either alone to the groups 4 and 5 (n = 10) or in combination to the group 6 (n = 10). Haloperidol (0.5 mg/kg) was injected 30 min before bromocriptine mesylate (5 mg/kg) when they are combined. Group 7 (n = 10) was used as vehicle control for the drugs injected.

Then, the rats were observed for 5 min at 30 min, 2, 4, and 6 h of the withdrawal period. At each observation session rats were assessed simultaneously for the following comprehensive behavioral conditions: horizontal, vertical, and ambulatory locomotor activity (Opto Varimex Minor, Columbus, OH), body posture, gait, agitation, tail stiffness, tremor, stereotyped behavior, wet dog shakes, and catatonia.

Both catatonia and wet dog shakes were expressed as incidence. Wet dog shake behavior was considered significant if it occurred at least three times during the observation period. Catatonia was evaluated by a vertical wire test (11). A 15-min immobilization in the vertical position was regarded as catatonia. The other behavioral parameters were scored in a range from 1 to 5 and the intensity of the parameters was expressed by median value.

At the 6th hour of the withdrawal, audiogenic stimulus (100 dB) was given to rats with a cutoff time of 1 min (10) following the completion of the behavioral evaluation. Then the incidence and the latency of the seizures were recorded.

Total EWS score was calculated by the following procedure: The behavioral parameters which were expressed as percent were scored in a range from 1 to 5 (10–20% : 1; 30–40% : 2; 50–60% : 3; 70–80% : 4; 90–100% : 5). Then the median values of each behavior were summed up for an individual observation period. Total sum was regarded as total EWS score.

Naive Control Experiments

Bromocriptine mesylate (5 mg/kg IP) and vehicle were also administered in two groups of naive Wistar rats (n = 6). Then horizontal, vertical, and ambulatory locomotor activities were recorded by the same method during the first 6 h after injection.

Statistical Analysis

Statistical significance was set at the p = 0.05 level. Comparison of withdrawal symptoms expressed as scores and incidence were done using the Mann-Whitney U-test and chi-square test, respectively. Changes in locomotor activities and latencies of audiogenic seizures were analyzed by the Student's t-test.

RESULTS

Ethanol Consumption and Blood Ethanol Level

Daily ethanol consumption of the rats was in a range of 12–17 g/kg. Blood ethanol level was found to be 270.22 ± 19.03 mg/dl (mean ± SE) at the beginning of the withdrawal period. It was measured as 42.52 ± 26.80 and 3.71 ± 0.54 mg/dl at 6th and 24th h of EWS, respectively.

Behavioral Changes During the First 6 h of EWS

Locomotor hyperactivity was observed in horizontal and ambulatory axis while vertical activity remained unchanged (Table 1). The hyperactivity was prominent at the 2nd h of the withdrawal and subsided through the 6th h. At the same time period, abnormal gait and posture, agitation, wet dog shakes, tail stiffness, tremor, stereotyped behavior, and catatonia were evaluated. Although abnormal gait and posture, trem-

TABLE 1

THE EFFECTS OF BROMOCRIPTINE ON HORIZONTAL, VERTICAL, AND AMBULATORY LOCOMOTOR ACTIVITY IN ETHANOL DEPENDENT AND NONDEPENDENT RATS (MEAN ± SE)

		Observation Intervals (h)			
		1/2	2	4	6
Vehicle (−)	H	406.166 ± 133.995	267.833 ± 92.088	332.833 ± 102.299	151.500 ± 42.447
(n = 6)	V	21.000 ± 10.298	31.833 ± 25.859	22.333 ± 6.014	5.833 ± 3.590
	A	262.00 ± 95.980	141.000 ± 62.184	186.666 ± 73.797	59.166 ± 18.847
Vehicle (+)	H	343,266 ± 56.817	558.449 ± 80.097*	551.850 ± 69.581*	398.684 ± 53.748
(n = 6)	V	16.933 ± 5.715	36.950 ± 9.449	28.700 ± 12.486	30.526 ± 12.846
	A	190.533 ± 40.772	364.450 ± 59.714*	322.099 ± 53.047*	218.157 ± 39.170
BRM 5 mg/kg (+)	H	240.000 ± 30.326	517.900 ± 70.421	607.000 ± 59.332	497.700 ± 80.922
(n = 10)	V	10.800 ± 4.368	54.000 ± 15.724	54.800 ± 11.860	40.600 ± 11.578
	A	110.200 ± 22.046	312.400 ± 53.593	356.400 ± 45.721	276.400 ± 53.082
BRM 5 mg/kg (−)	H	473.166 ± 85.878	1129.333 ± 255.846†‡	1281.333 ± 199.081†‡	1462.500 ± 277.968†‡
(n = 10)	V	50.000 ± 11.943	209.000 ± 46.565†‡	300.166 ± 67.148†‡	328.166 ± 82.660†‡
	A	286.500 ± 62.138	854.000 ± 233.197†‡	969.666 ± 180.638†‡	1145.000 ± 238.533†‡

h: hour; −: ethanol nondependent; +: ethanol dependent; H: horizontal; V: vertical; A: ambulatory; BRM: bromocriptine.
*p < 0.05, significantly different from 1/2th h.
†p < 0.01, significantly different from vehicle (−).
‡p < 0.01, significantly different from BRM (+).

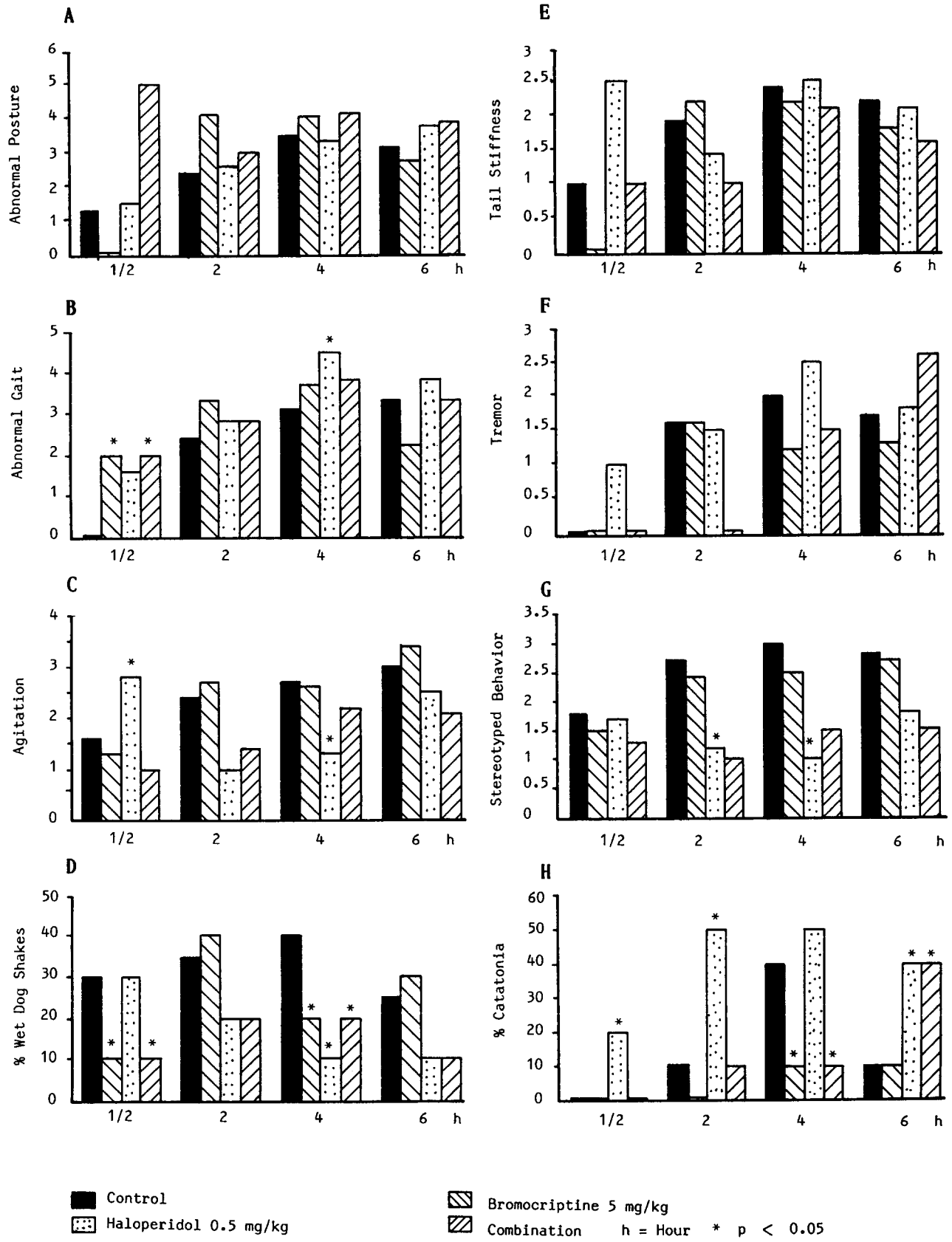

FIG. 1. Effects of bromocriptine, haloperidol, and their combination on EWS symptomatology.

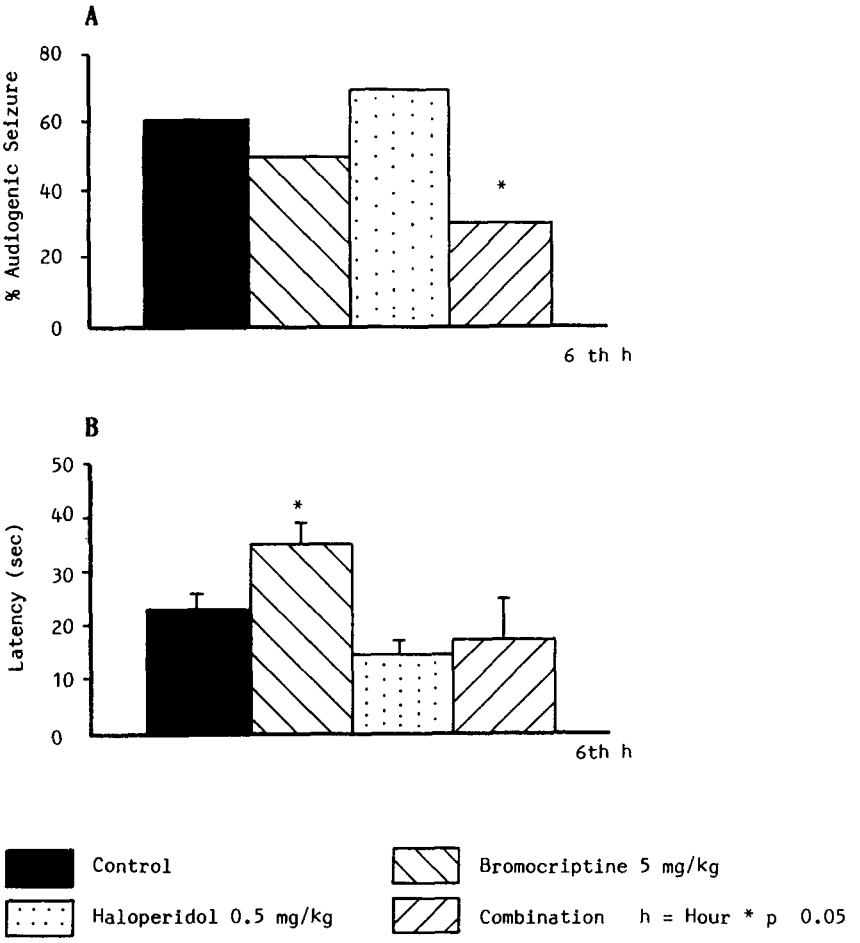

FIG. 2. Effects of bromocriptine, haloperidol, and their combination on the incidence (A) and latency (B) of audiogenic seizures during EWS.

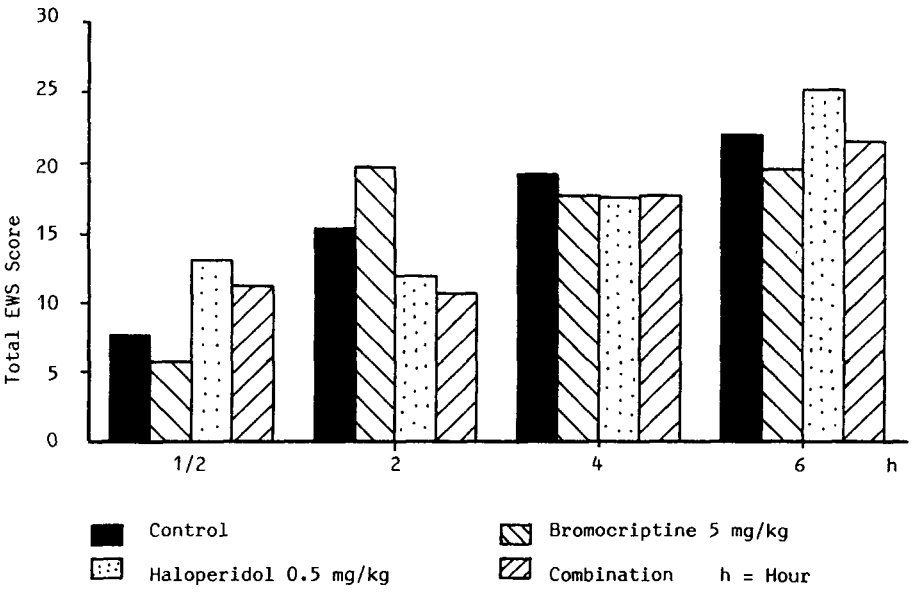

FIG. 3. Effects of the drugs on the time course of the total EWS scores.

ors, tail stifness, and catatonia progressively increased in intensity through the 6th h, the other behavioral events were maintained in a relatively high level after the initial observation until the end. The time course of the total score of EWS is shown Fig. 1 (A–H, dark bars).

Effects of the Drugs on the First 6 h of EWS

The incidence of wet dog shakes and catatonia were reduced by bromocriptine (5 mg/kg IP) but the intensity of abnormal gait was potentiated. The other behavioral changes including locomotor hyperactivity were not affected by bromocriptine injection (Table 1; Fig. 1).

Haloperidol (0.5 mg/kg SC) reduced the incidence of wet dog shakes and the intensity of stereotyped behavior but potentiated abnormal gait, agitation, and catatonia (Fig. 1). Furthermore, haloperidol precipitated two additional events, teeth chattering and spontaneous convulsions, which did not occur during control EWS and inhibited locomotor hyperactivity during the first 6 h of EWS (data not shown).

Haloperidol failed to antagonize the inhibitory effect of bromocriptine on the incidence of wet dog shakes and catatonia. The potentiatiating effect of bromocriptine on abnormal gait was not antagonized by haloperidol, but bromocriptine reduced the effects of haloperidol on agitation and stereotyped behavior (Fig. 1).

Audiogenic Seizures and Effects of the Drugs

Exposure to audiogenic stimulus at the 6th hour of the withdrawal precipitated seizures with an incidence of 60% and a latency of 24.0 ± 4.43 s (Fig. 2A and B). No mortality was recorded during seizures.

Bromocriptine increased the latency of the seizures, but their incidence remained unchanged. Haloperidol was found to be ineffective on audiogenic seizures. The incidence of the seizures was decreased significantly when haloperidol and bromocriptine were given in combination (Fig. 2 A).

Total EWS Score

Bromocriptine and haloperidol either alone or in combination did not alter the total EWS score (Fig. 3).

Effect of Bromocriptine on Naive Rats

Bromocriptine (5 mg/kg IP) produced in naive rats a locomotor hyperactivity in horizontal, vertical, and ambulatory axis beginning at 2nd hour after injection, which was significantly higher than that observed during EWS (Table 1).

DISCUSSION

The signs of EWS as observed in the present work indicated progressive CNS stimulation. The increased excitation manifests itself with a rich behavioral symptomatology including audiogenic seizures (9,21).

Some behavioral changes occurring in EWS such as horizontal hyperactivity, stereotyped behavior, tremors, and catatonia have implicated a possible contribution of the brain dopaminergic system in the development of EWS symptomatology because all these behaviors can be induced by dopaminergic receptor agonists or antagonists.

Bromocriptine did not potentiate the behavioral changes that may be induced by dopaminergic hyperactivity during EWS (Table 1 and Fig. 1), yet, the same dose of bromocriptine produced more potent locomotor hyperactivity in naive rats (Table 1). This observation is consistent with the hypothesis of dopaminergic hypoactivity supported by the results indicating dopaminergic receptor subsensitivity (16,18) or decreased dopamine turnover (5) during EWS. On the other hand, a dopaminergic hyperactivity hypothesis was supported by the findings that haloperidol inhibited the stereotypic behavior and agitation. This discrepancy may be explained by an heterogenous alteration of the dopaminergic activity in the brain regions during EWS.

It has been reported that increased seizure sensitivity in EWS is another component of the progressive CNS excitability (9). Our results also indicate that the brain dopaminergic system might contribute to the development of seizures. Although bromocriptine inhibited wet dog shakes behavior, which is a preconvulsive sign (Fig. 1D), haloperidol increased the incidence of the spontaneous convulsions (data not shown). Bromocriptine prolonged the latency of the audiogenic seizures that was evaluated at the 6th hour of the withdrawal period. Haloperidol antagonized the inhibitory activity of bromocriptine on latency of audiogenic seizures (Fig. 2B). These findings may suggest the involvement of dopamine D_2 receptor-related events in the increased latency of the audiogenic seizures.

It has been reported that bromocriptine administration decreased the incidence of audiogenic seizures in EWS (20). Our results (Fig. 2A) did not support this finding obtained with 2.5 mg/kg IP bromocriptine. A possible reason for this discrepancy might be related to the difference in dose of bromocriptine and time of exposure to audiogenic stimulus.

It was also demonstrated by the present study that bromocriptine and haloperidol either alone or in combination are not effective on the severity of the total EWS score (Fig. 3).

In conclusion, the brain dopaminergic system may be involved to a limited extent and in a complex fashion in the causation of EWS, and this involvement may explain various behavioral changes recorded during EWS in the rats.

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