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Effect of prenatal administration of haloperidol, risperidone, quetiapine and olanzapine on spatial learning and retention in adult rats

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Abstract

The typical antipsychotic haloperidol and atypical antipsychotics olanzapine, quetiapine and risperidone were administered to pregnant Sprague–Dawley dams in the drinking water from Days 8 to 18 of gestation. When the offspring reached adulthood (2 months), spatial learning and short-term retention were examined using the radial arm maze. Results showed that prenatal administration of haloperidol, risperidone and quetiapine impaired learning but only haloperidol and risperidone disrupted short-term retention. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

Pre- and postnatal administration of agents which alter dopaminergic (DA) neurotransmission can disrupt the normal development of the DA system in the offspring. For example, prenatal exposure to a number of neuroleptics can reduce cell proliferation in the brain (Blackhouse et al., 1982; Patel and Lewis, 1988), decrease D1 and D2 receptor densities in the caudate and n. accumbens (Scalzo et al., 1989; Williams et al., 1992), attenuate DA autoreceptor function (Scalzo and Spear, 1985) and alter responses to DA agonists and antagonists (Cuomo et al., 1985; Scalzo et al., 1989; Spear et al., 1980). Neonatal depletion of DA with 6-OHDA has been reported to increase sensitivity to DA agonists and antagonists (Breese et al., 1987; Gong et al., 1993; Huang et al., 1997; Neal-Beliveau and Joyce, 1991). A number of behavioral abnormalities have been reported in adult rats following both prenatal administration of neuroleptics (principally haloperidol) and neonatal depletion of DA. These include impairment of radial maze learning (Archer, 1993) interference with differential reinforcement of low rate (DRL) operant performance (Cuomo, 1981), disruption of Morris water maze learning and impaired latent learning (Archer, 1993; Archer and Fredrikson, 1992). Little information is, however, available on the effects of prenatal administration of the newer atypical antipsychotics on cognitive performance in adult animals. The purpose of the present experiment, therefore, was to examine the consequences of prenatal administration of olanzapine, quetiapine and risperidone on spatial learning and retention in adult rats and to compare their effects with those of haloperidol. Since psychotropic medications are commonly prescribed for women of childbearing age (Altshuler et al., 1996), the identification of an antipsychotic with minimal effects on cognition is of particular importance.

2. Experimental procedure

2.1. Animals and treatments

Thirty-timed pregnant Sprague–Dawley rats, 350 g of weight at the beginning of the study, were administered vehicle, haloperidol, quetiapine, olanzapine and risperidone between gestation days (GD) 8 and 18. A separate group of pregnant dams treated with vehicle, to which 0.1% of saccharin was added, was designated to be used for the cross fostering procedure. Drugs were administered in the drinking

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Table 1
Water intake during GD 8–18 and antipsychotic treatment

Treatment	Water intake (11 days)	Weight gain (11 days)	*N* of litters	Total *N* of pups	Pup weight at 24 h PN	Pup weight at 21 days PN	Pup weight at 42 days PN
Vehicle	594±24	72±2.0	6	62	6.58±0.21	57±2.36	187.00±3.70
Haloperidol	565±30	66±1.5*	6	60	7.33±0.24	58±2.77	190.41±2.32
Olanzapine	551±34	68±2.4*	6	62	6.91±0.09	56±1.78	169.58±3.26*
Risperidone	701±38*	67±1.6*	6	64	6.75±0.13	53±1.31	157.33±3.15*
Quetiapine	676±41*	71±2.0	6	61	6.75±0.18	50±1.60	149.41±3.82*
One-way ANOVA	F=12.83, P < .0001	F=25.47, P < .0001					F=29.22, P < .0001

The water intake was significantly higher in risperidone- and quetiapine-treated pregnant dams [one-way ANOVA, F=12.83, P < .0001]. Weight gain during gestation was significantly lower in haloperidol-, olanzapine- and risperidone-treated rats [one-way ANOVA, F=25.47, P < .0001]. Weight of 42-day-old in utero exposed pups was significantly lower in olanzapine, risperidone and quetiapine groups [one-way ANOVA, F=29.22, P < .0001].

water. Saccharin (0.1%) was added to the vehicle and drug solution. The water intake was not restricted. The amount of water consumed by the pregnant rat was measured daily during 11 days of drug administration. Drug concentration and water consumption were determined and adjusted daily according to animal weight gain and water consumption. The doses of haloperidol and risperidone were chosen to block over 70% of the D2 dopamine receptors, which is the usual therapeutic goal. The doses of quetiapine and olanzapine were selected so as to be equivalent in clinical potency to haloperidol. Haloperidol and olanzapine were administered in the drinking water 2 mg/kg/day, risperidone 1 mg/kg/day and the dose of quetiapine 10 mg/kg/day. Doses of the antipsychotics were adjusted daily to deliver the amount of the antipsychotic in the drinking water as close as possible to the selected dose (Tables 1 and 2). Maternal weight gain and water intake was recorded. At birth, male and female pups from each treated litter were cross-fostered to untreated lactating dam and the number of pups was culled to 10 per litter. Rats were weaned at 25 days of age, were separated (males and females) and were then group-housed in wire cages (two rats per cage). Only male pups were used in this study. Pup weights were recorded at birth and at the ages of 1, 3, 7, 14, 21 and 42 days. When rats reached 2 months of age, two male rats from each vehicle- and drug-treated litter (total of 12 pups per group) were weighed and introduced to a restricted diet designated to maintain their weight at 85% of ad libitum levels. Spatial learning and retention was studied in a radial arm maze. For statistical analysis, data from two rats from each litter were averaged and considered as an *n* of 1.

Table 2
Daily doses of haloperidol, olanzapine, risperidone and quetiapine administered between GD 8 and 18

Day of treatment	Haloperidol	Olanzapine	Risperidone	Quetiapine
1	1.82±0.03	1.88±0.05	0.95±0.02	9.68±0.3
2	2.00±0.04	2.22±0.04	1.16±0.02	11.68±0.4
3	2.13±0.04	2.23±0.07	1.12±0.03	10.28±0.1
4	2.18±0.03	2.08±0.06	1.08±0.02	9.92±0.1
5	1.88±0.02	1.96±0.07	1.17±0.02	10.86±0.2
6	2.01±0.05	2.18±0.05	1.07±0.02	10.26±0.2
7	2.10±0.06	1.89±0.02	1.14±0.03	10.29±0.2
8	2.21±0.04	2.01±0.08	1.18±0.04	9.48±0.1
9	1.89±0.03	1.82±0.01	1.19±0.02	9.55±0.2
10	2.07±0.04	1.88±0.03	1.13±0.01	9.20±0.2
11	1.94±0.05	1.92±0.01	1.00±0.01	9.16±0.1

Values are expressed in mg/kg/day.

2.2. Apparatus

Apparatus was an eight-arm maze constructed from wood. Each arm was 60 cm long, 12 cm wide with a recessed food well 2.5 cm in diameter at the end of each arm. The central area was an octagonal shaped hub, 40 cm in diameter. Clear Plexiglas guillotine doors, operated by a remote pulley system, controlled access to the arms. The maze was elevated 80 cm above floor level and situated in a room in which several distinctive objects of constant location served as extra maze cues.

2.3. Procedure

Animals were first adapted to the apparatus in three daily 7-min sessions. Fruit loop cereals were sprinkled throughout the maze. Doors remained in the open position and rats were permitted to explore the maze and eat the cereal.

On Day 4, doors were lowered and raised when rats entered and left each arm so that they would be familiar with the procedure used in training. A training sessions was begun by baiting all eight arms. Each rat was then placed into the central arena and all of the doors were raised. When the rat entered an arm the doors were lowered. When the reward was consumed the door was raised allowing the rat to return to the central arena. After a 5-s interval, the next trial was initiated by again raising all of the doors simultaneously. This procedure was continued until the animal had entered all arms or until 7 min have elapsed. Daily acquisition sessions were continued until each rat achieved a criterion of three consecutive sessions with one error or less. An error was defined as a reentry into a previously visited arm. When rats achieved the acquisition criterion, tests were begun to determine the temporal gradient of working memory. Each rat was permitted to make four arm choices and was then returned to the home cage for a duration of 5, 60 or 120 min. Rats were assigned to the various intervals using a modified Latin Square procedure.

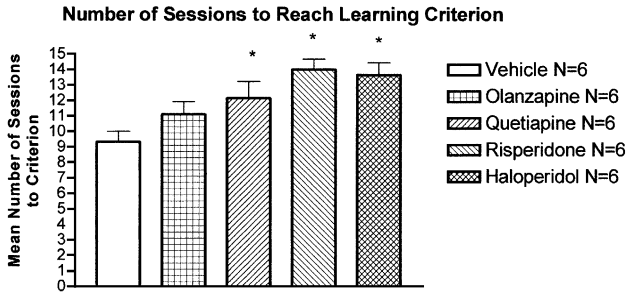

Fig. 1. The effect of prenatal exposure of rat pups to treatment with vehicle, olanzapine, quetiapine, risperidone and haloperidol on spatial learning in eight-arm radial maze. Pups exposure in utero to quetiapine, risperidone, risperidone and haloperidol required significantly more sessions to reach the learning criterion than olanzapine- and vehicle-treated rats. A one-way ANOVA computed for these data reveals a significant difference among five treatment groups [$F(4,25) = 6.97$; $P < .001$].

At the end of the test intervals, rats were returned to the maze and the number of errors to enter the four remaining arms was determined. All animals received two trials at each interval.

2.4. D1 and D2 dopamine receptor binding procedure

Rats were sacrificed, brains were quickly removed caudates dissected and stored at −80 °C until assay. For the binding assay, striatal tissue (for D1 and D2 binding) and prefrontal cortex tissue (for D1 binding) was homogenized with a Brinkman Polytron for 5 s at setting 6, in 0.05 M Tris buffer containing 1 mM EDTA, 5 mM KCl, 1.5 mM $CaCL_2$, 4 mM $MgCl_2$, 12 mM NaCl, pH 7.4. Homogenates were centrifuged at 20,000×g for 10 min and the membrane pellets were resuspended in same buffer to yield the final tissue concentration of 4 mg/ml. D1 and D2 dopamine receptors were assayed using 0.5 nM concentration of 3H-SCH 23390 (S.A. 70.0 Ci/mmol, Amersham) and 0.2 nM concentration of 3H-spiroperidol (S.A. 24.0 Ci/mmol, New England Nuclear) according to the method of Billard et al. (1984) and List and Seeman (1981), respectively. Tubes were incubated for 30 min at 37 °C and filtered through Whatman GF/B filters and washed three times with the same buffer, using a Brandel Cell Harvester. Radioactivity in the filters was estimated by scintillation spectroscopy.

3. Results

The drug/water intake revealed that there was no difference among vehicle-, haloperidol- and olanzapine-treated pregnant rats whereas risperidone and quetiapine demonstrated higher fluid intake One-way ANOVA demonstrated statistically significant difference [F(12,83); $P < .0001$] (Table 1). Maternal weight gain during gestation was higher in quetiapine- and risperidone-treated pregnant dams. One-way ANOVA revealed statistically significant difference [$F = 25.47$; $P < .0001$] (Table 1). Size of the litter was not affected by drug administration during gestation and there were no statistically significant weight differences among the groups at birth and at the age of 21 days (Table 1). Rats treated in utero with vehicle, haloperidol, olanzapine and risperidone demonstrated gradual significant weight gain. However, in risperidone, olanzapine and quetiapine groups, the weight gain was slower (Table 1). One-way ANOVA revealed statistically significant differences [$F = 29.22$; $P < .0001$)

Fig. 1 shows the mean trials to reach a learning criterion of three daily trials with one error or less. A one-way ANOVA computed for these data reveals a significant difference among the five treatment groups [$F(4,25) = 6.97$, $P < .001$].Comparison of each group with the vehicle control using Newman–Keuls tests revealed that all treatment groups except olanzapine required significantly more sessions to reach the learning criterion than vehicle controls.

Effect of prenatal drug treatment on retention over the three delay intervals is shown in Fig. 2. Results of a 3×5

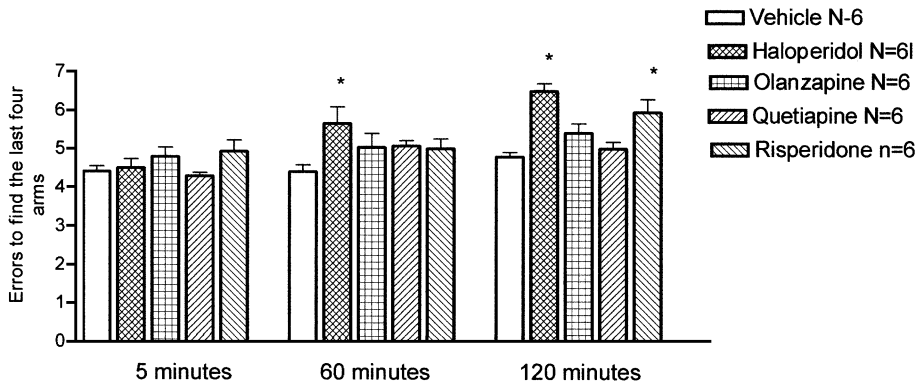

Fig. 2. Effects of prenatal exposure of rat pups to treatment with vehicle, olanzapine, quetiapine, risperidone and haloperidol on retention over three delay intervals. Results of 3×5 ANOVA indicate significant main effect for treatment groups [$F(4,25) = 6.97$; $P \leq .001$] and a significant main effect for delay intervals [$F(2,50) = 18.73$; $P < .0001$]. The interaction effect was significant [$F(2,50) = 18.73$; $P < .0001$]. The interaction effect was significant [$F(8,50) = 2.3$; $P = .0342$]. Multiple comparisons using Dunnet's test demonstrated that at 60-min interval, only haloperidol impaired retention while at 120-min interval, risperidone and haloperidol impaired retention. Olanzapine and quetiapine had no effect on retention.

ANOVA carried on these data indicated a significant main effect for treatment group [$F(4,25) = 6.97$; $P \leq .001$] and a significant main effect for delay interval [$F(2,50) = 18.73$; $P < .0001$]. The interaction effect was significant [$F(8,50) = 2.3$; $P = .0342$].

Multiple comparisons using Dunnett's test demonstrated that at 60-min interval only haloperidol impaired retention ($P < .05$) while olanzapine and quetiapine had no effect. At 120-min interval, haloperidol and risperidone impaired retention ($P < .05$) while olanzapine and quetiapine had no effect. There were no statistically significant differences in striatal D1 and D2 receptor binding or in prefrontal D1 receptor density at the age of 4 months (data not shown).

4. Discussion

These results indicate that acquisition of spatial information in adulthood is disrupted by prenatal administration of the typical antipsychotic haloperidol and the atypical antipsychotics quetiapine and risperidone. The disruptive effects of haloperidol on spatial learning confirms a previous report by Archer (1993) but there has been little prior evidence to suggest that the newer atypical antipsychotics can also impair learning. There were no significant group differences in latency to visit all eight arms during acquisition so that it is unlikely that alterations in activity level could account for the slower rate of learning in haloperidol, quetiapine and risperidone groups. The results of interpolating a delay between the first and last four choices indicates that prenatal haloperidol and risperidone administration also disrupted memory processes. This is clear from Fig. 2, which shows that performance of the haloperidol and risperidone-treated group is impaired only at the longer retention intervals. Although prenatally administered quetiapine and risperidone impaired acquisition of spatial learning, quetiapine had no significant disruptive effect on retention. It is noteworthy that prenatally administered olanzapine failed to disrupt learning or retention in this study.

The in vitro profile of the atypical and typical antipsychotics may be responsible for the differences in their ability to induce deficits in learning and memory. Antipsychotics affect cholinergic, adrenergic, serotonergic and DA neurotransmitter systems and have different affinity in vitro for the corresponding receptors (Schotte et al., 1993; Bymaster et al., 1996). D1 and D2 receptors and cholinergic receptors are involved in cognitive processes (Savagushi and Goldman-Rakic, 1991; Arnsten, 1997; Keefe et al., 1999; Murphy et al., 1996; Lee et al., 1999). Thus, variation in the different receptor affinities provide a potential mechanism which might account for the pattern of results reported in this experiment. Olanzapine has lower affinity for the D2 receptor than haloperidol and risperidone and is also bound more loosely to the receptor (Bymaster et al., 1996; Seeman and Tallerico, 1999). It is, therefore, plausible to suggest that antipsychotics which have low affinity for the dopamine receptors may be more readily displaced by endogenous DA. Better access of dopamine to the maturing receptors may reduce the potential for cognitive disruption in the offspring.

Different mechanism, however, may be responsible for the effect of quetiapine and risperidone observed in this study. Quetiapine in contrast to risperidone and olanzapine has a very low affinity for dopamine receptors and is a potent antiserotonergic drug. In embryonic rat brain, serotonin acts as a differentiation signal for serotonin neurons and receptors during sensitive periods for rat brain development (Lauder and Krebs, 1999; Lauder et al., 2000; Whitaker-Azmitia et al., 1987). During this period pregnant dams were administered quetiapine in our study. It is, therefore, also plausible that the effects of risperidone are related not only to its anti-DA but also to the potent antiserotonergic effects. This study has demonstrated that administration of antipsychotics during sensitive periods of rat brain development can have prolonged effects on cognitive function.

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