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Dopamine-Dependent Nature of Depression-Like Behavior in WAG/Rij Rats with Genetic Absence Epilepsy

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WAG/Rij rats given placebo showed a depression-like state as compared with normal Wistar rats (lacking convulsive pathology); this was analogous to the state previously seen in rats of this line, with decreased investigative activity in the open field test, increased immobility in the forced swimming test, and decreased consumption and preference for sucrose solution (anhedonia). Chronic administration of the tricyclic antidepressant imipramine (15 mg/kg, i.p., 15 days) had therapeutic (antidepressant) effects on depression-like behavior in WAG/Rij rats. After withdrawal of antidepressant therapy, the behavior of WAG/Rij rats was not significantly different from that of Wistar rats. Acute (single-dose) administration of the selective dopamine D_2/D_3 receptor antagonist raclopride (100 μg/kg, i.p., 15 min before the start of behavioral testing) increased the symptoms of depression-like behavior and suppressed the antidepressant effect of chronic administration of imipramine in WAG/Rij rats. Raclopride had no significant effect on behavior in Wistar rats. Administration of the dopamine D_2/D_3 receptor agonist parlodel (a therapeutic form of bromocriptine) cured the depression-like behavior of WAG/Rij rats and had no significant effect on behavior in Wistar rats, with the exception of a reduction in the duration of immobility in the forced swimming test. Imipramine and raclopride had no significant effect on the levels of total movement activity and anxiety in either WAG/Rij or Wistar rats. These results demonstrate the dopamine-dependent nature of depression-like behavior in WAG/Rij rats and show the possible involvement of dopamine D_2 receptors in mediating the antidepressant effect of imipramine on genetically determined depression-like behavior in WAG/Rij rats.

KEY WORDS: depression-like behavior, WAG/Rij rats, absence epilepsy, Wistar rats, antidepressant treatment, imipramine, raclopride, dopamine D_2/D_3 receptor antagonist, dopamine D_2/D_3 receptor agonist.

Previous studies have established that one of the characteristics of the inbred rat line WAG/Rij is the presence of spontaneous peak-wave discharges on the EEG, these being reminiscent of absence epilepsy in humans [9]. Subsequent studies showed that WAG/Rij rats, as compared with normal Wistar rats (lacking convulsive pathology), show depression-like behavior, with decreased investigative activity in the open field test, increased immobility in the forced swimming test, and decreased sucrose consumption [4–6, 24, 25]. The phenomenological similarity of the behavioral characteristics of WAG/Rij rats with depressive pathology in humans (face validity) and the need for courses of treatment (i.e., chronic treatment) to obtain a therapeutic (antidepressant) effect with imipramine (in the Porsolt test) in WAG/Rij rats, just as in depressed patients (predictive validity), indicate that this rat strain can be regarded as a new experimental model of depression [4, 6, 24]. A large amount of factual material has now accumulated to provide evidence of the key role of brain dopamine, particularly dopamine D_2/D_3 receptors, in the pathogenesis of depressive impairments in animals and humans [8, 12] and in mediating the therapeutic effects of antidepressants, regardless of their neurochemical mechanisms of action [11, 12,

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20, 22, 30]. Previous behavioral and electrophysiological studies have demonstrated functional deficits in the brain dopaminergic system in WAG/Rij rats [6, 15, 18]. It is significant that dysfunction of the brain dopaminergic system induced in normal Wistar rats with a specific dopaminergic neurotoxin led to the appearance of a complex of symptoms of depressive behavior [3] corresponding completely to the genetically determined depression-like behavior in WAG/Rij rats. These data led to the suggestion that the depression-like behavior of WAG/Rij rats may be dopamine-dependent in nature. The aim of the present work was to test this suggestion.

METHODS

Animals. Studies were performed using male WAG/Rij rats (n = 33) obtained from Holland in 1995 and maintained at the Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, as well as male Wistar rats (n = 32) from the Stolbovaya animal supplier. All animals were kept in standard animal-house conditions in groups of 5–6 per cage with free access to food and water and with a natural day:night cycle (with a day length of about 10 h). Before studies, WAG/Rij and Wistar rats were exposed to a sound stimulus ("the sound of keys") to assess their predisposition to audiogenic convulsions (the suitability of this method has been described previously) [15, 19]. In the present studies, as in our other investigations [4, 6, 24], only those animals not predisposed to audiogenic convulsive seizures, i.e., WAG/Rij rats (n = 33) with "pure" absence epilepsy and Wistar rats (n = 32) completely lacking convulsive pathology, were used. Studies were performed in accord with the regulations for working with animals and the experimental protocols were approved by the Ethics Commission of the Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences.

Experimental series. In the first series of experiments, WAG/Rij rats were used to assess the possible suppression of the therapeutic (antidepressant) effect of imipramine using the selective dopamine D_2/D_3 receptor antagonist raclopride. The second series of experiments addressed the possibility of eliminating depression-like behavioral characteristics in WAG/Rij rats using the dopamine D_2/D_3 receptor agonist parlodel (a therapeutic form of bromocriptine). Experiments were performed from 10:00 to 15:00.

Behavioral tests. Behavioral differences between different groups of WAG/Rij and Wistar rats (placebo-treated controls and experimental groups given study agents) were identified using standard tests for assessing the level of anxiety (the open field test, light-dark selection) [10, 27] and depressivity (sucrose consumption test, sucrose preference test, forced swimming test) [17, 21]. The anxiogenic influences of experimental procedures such as handling [26], being placed in the transfer cage, and transportation from the animal house to the experimental room and back were minimized by adapting the animals for five days before the behavioral experiments started.

TESTS FOR ASSESSMENT OF THE LEVEL OF ANXIETY

Open field test. The open field was a round arena of diameter 100 cm surrounded by a wall 30 cm high and divided into 32 squares. The four central squares were regarded as the "center" of the field. The rat was placed in the center of the field, in which the illumination level decreased from 80 lx in the center to 20 lx at the walls. The following parameters were recorded during the 10-min test: the latent period of the first movement (sec), the number of squares crossed (movement activity), the numbers of rearings and excursions to the center of the field (investigative activity), the number of grooming reactions, and the number of boluses. Lower activity in the open field test is usually taken as a measure of a higher level of anxiety and vice versa [13].

The light–dark selection test. The light–dark selection test used a closed metal chamber divided into two communicating sectors: a large sector (36 × 18 cm), which was illuminated (100 lx), and a small sector (18 × 17 cm), which was dark (<5 lx). The rat was placed in the light part of the chamber with the tail towards the entrance of the dark sector. The latent period of the excursion into the dark sector was measured during the 5-min test, along with the times spent in the light and dark sectors, the number of transfers between sectors, the number of rearings in the light sector, and the number of "risk" assessments, where the rat glanced from the dark sector or partly left it for the light sector, after which it rapidly hid itself in the dark sector. As a rule, the less time spent in the light sector, the smaller the number of transfers from one sector to the other; the larger the number of "risk" assessments, the higher the level of anxiety, and vice versa [7, 10].

TESTS FOR ASSESSMENT OF THE LEVEL OF DEPRESSIVITY

Sucrose consumption and preference test. In the sucrose consumption test, the rat was placed in the experimental chamber for 15 min and, after stabilization of the consumption level, the total quantity (g) of 20% sucrose solution drunk was measured, along with the mean quantity of sucrose drunk during each approach to the bowl (a measure of the "tastiness" of sucrose, i.e., sucrose liking, palatability) [14] and the number of approaches to the bowl (an indirect measure of movement activity). Sucrose con-

sumption was measured as the difference in the weight (g) of the bottle before and after the test. Animals were not subjected to any special food or drink deprivation procedure but underwent preliminary adaptation to the chamber. In the sucrose preference test, the rat was placed in the same experimental chamber used for the sucrose consumption test and was presented with two bottles: one with 20% sucrose solution and one with plain water. The quantities of sucrose and water consumed over a period of 1 h were measured. The preference for sucrose over water (PS, %) was calculated as PS = [(sucrose consumed, g/total liquid consumed (sucrose + water), g) × 100] [17]. Testing for sucrose preference was performed after 23-h food and drink deprivation. The quantity of sucrose drunk is an indicator of hedonism widely used for assessing the sensitivity of animals to reinforcements evoking the feeling of satisfaction [17, 20, 28]. Decreased sucrose consumption, or anhedonia, is regarded as a reliable measure of the depression state in animals [29].

The forced swimming test. This test is one of the most widely used tests for evaluating the level of depressivity in animals and assessing the antidepressant activity of pharmacological agents [21, 23]. The forced swimming test performed here was based on a cylindrical plastic tank (height 47 cm, internal diameter 38 cm) filled with water to a depth of 38 cm. The water temperature was maintained at 22 ± 1°C. The rat was placed in the water and the durations of passive swimming (immobility), the first episode of active swimming (climbing), and swimming (the total duration of active swimming excepting the duration of the first episode of active swimming) were measured, along with the numbers of dives and boluses. The greater the duration of immobility, the shorter the duration of swimming and the shorter the duration of the first episode of active swimming, the greater the level of depressivity, and vice versa. The test lasted 5 min.

Pharmacological agents. The first series of experiments used the tricyclic antidepressant imipramine HCl (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) and the dopamine D_2/D_3 receptor antagonist raclopride (S(–)-raclopride-L-tartrate, Tocris Cookson Inc., Ellisville, MO, USA). Imipramine was used in distilled water and was given i.p. at a dose of 15 mg/kg/day for 15 days [16]. Raclopride was dissolved in distilled water and given as single i.p. doses of 100 μg/kg 15 min before the start of tests [20]. Control animals were given placebo (the solvent used for the agent). WAG/Rij rats (WR, n = 21) and Wistar rats (WS, n = 20) were randomized to two groups (WS1, WS2, WR1, WR2). The first groups (WS1, WR1) were given chronic (15 days) placebo; the second group (WS2, WR2) received imipramine. Each group was then divided into two subgroups (1A, 1B, 2A, 2B), one of which (1A, 2A) was given placebo 15 min before behavioral testing, the other (1B, 2B) being given raclopride. Raclopride was given once to the same animals 15 min before the start of the test. Each animal was then tested sequentially in the light-dark selection test, the open field test, the sucrose consumption test, and the forced swimming test (35 min). The sucrose preference test was performed for 1 h the next day, after 23-h food and drink deprivation, in 5–6 rats (in different cages) simultaneously (total time 120 min). The effects of chronic administration of imipramine are known to last 48 h. All data from these tests performed in each animal were obtained at <48 h from the start of imipramine dosage. The second series of experiments used parlodel, a therapeutic form of bromocriptine, which is a dopamine D_2/D_3 receptor agonist (bromocriptine mesylate, Novartis Pharma Services Inc., Basel, Switzerland). Parlodel was give p.o. as a suspension at a dose of 5 mg/kg in a volume of 1 ml/kg body weight for 15 days. Suspensions were prepared immediately before administration by dissolving ground tablets in 1–2 drops of Tween-80 followed by dilution to the required volume with physiological saline [1]. WAG/Rij rats (n = 12) and Wistar rats (n = 12) were randomized to two groups (six in each). Experimental animals received parlodel; controls received solvent. All substances were given in a volume of 1 ml/kg body weight. Behavioral testing was performed after chronic treatment with agent lasting 15 days (first series) and 15 days after withdrawal of treatment (second series).

Statistical analysis of data. Statistical data analysis was performed using the statistical program bundle Statistica 6.0. The effects of the "treatment" factor (imipramine, raclopride) and the "rat strain" factor (WAG/Rij, Wistar) were assessed by three-factor dispersion analysis (three-way ANOVA); the effects of treatments within each rat strain were assessed by two-way dispersion ANOVA. Mean values for the various measures were compared using Student's t test modified for unequal dispersions. Analysis of the data from the second series of experiments used, along with two-way analysis, single-factor dispersion analysis (one-way ANOVA).

RESULTS

First experimental series. In the first series of experiments, WAG/Rij (n = 21) and Wistar rats (n = 20) were used to investigate the possible suppression of the therapeutic (antidepressant) effect of imipramine by the dopamine D_2/D_3 receptor antagonist raclopride. In the light-dark selection test (Table 1), WAG/Rij rats given placebo (WR1A), as compared with the corresponding Wistar group (WS1A), showed no significant difference in behavior indicative of anxiety in terms of all measures other than a smaller number of rearings in the light sector of the chamber. The significance of the "rat strain" factor for the number of rearings in this test was high ($F(1.33)$ = 27.94, $p < 0.001$). Administration of imipramine, as compared with placebo, had no significant effect on behavior (level of

TABLE 1. Behavioral Measures in WAG/Rij and Wistar Rats in the Light–Dark Selection Test

Behavioral measure	Rat groups							
	WAG/Rij (WR)				Wistar (WS)			
	WR1A (n = 5)	WR1B (n = 5)	WR2A (n = 5)	WR2B (n = 6)	WS1A (n = 5)	WS1B (n = 5)	WS2A (n = 5)	WS2B (n = 5)
Latent period of entry into the dark sector, sec	28.0 ± 6.04	38.0 ± 4.89	25.0 ± 3.16	24.16 ± 5.39	38.4 ± 16.84	23.0 ± 5.83	62.0 ± 18.55	58.0 ± 17.07
Time spent in light sector, sec	88.4 ± 22.78	48.0 ± 8.6	72.0 ± 13.29	47.5 ± 9.20	98.4 ± 14.42	71.0 ± 24.21	99.0 ± 24.05	81.0 ± 21.47
Number of transfers between sectors	4.2 ± 1.02	2.0 ± 0.45	3.4 ± 0.51	2.8 ± 0.17	4.6 ± 0.75	3.8 ± 1.02	4.0 ± 0.89	3.2 ± 0.58
Number of rearings in light sector	**1.4 ± 0.60***	0.4 ± 0.24	1.2 ± 0.2	0.5 ± 0.34	3.2 ± 0.49	2.8 ± 1.20	4.4 ± 1.21	3.8 ± 0.73
Number of "risk" assessments	4.4 ± 0.6	**2.0 ± 0.32****	4.4 ± 0.51	3.3 ± 0.42	4.0 ± 0.45	5.6 ± 1.50	5.6 ± 1.48	5.0 ± 1.14

Notes. Mean values and errors of the mean (M ± SE) are given for each behavioral measure. Groups: 1A) placebo + placebo; 1B) placebo + raclopride; 2A) imipramine + placebo; 2B) imipramine + raclopride. $^{*}p < 0.05$ in WAG/Rij rats compared with Wistar rats, $^{++}p < 0.01$ for group 1B compared with group 1A (effect of raclopride). The significance of differences in mean measures on Student's t test modified for unequal distributions. Bold face shows significant differences between WAG/Rij and Wistar rats.

anxiety) in either WAG/Rij or Wistar rats: three-way dispersion analysis showed that $F(1.33) \leq 1.72$, $p \geq 0.20$ for all measures in this test. Administration of raclopride significantly decreased the number of transfers ($F(1.33) = 4.69$, $p = 0.037$) and the time spent in the light sector of the chamber ($F(1.33) = 4.36$, $p = 0.044$) in both WAG/Rij and Wistar rats. Two-way dispersion analysis showed that raclopride significantly decreased the number of transfers ($F(1.17) = 5.50$, $p = 0.03$) and the number of rearings ($F(1.17) = 5.04$, $p = 0.038$) only in WAG/Rij rats (in Wistar rats, $F(1.16) \leq 2.34$, $p \geq 0.14$ for all measures). However, post hoc comparison using Student's t test did not support the significance of the differences obtained in the two- and three-way dispersion analyses, which is evidence that the differences in these parameters of "anxiety" in WAG/Rij rats remained within the range expected on the basis of random variations. Furthermore, raclopride significantly decreased the number of "risk" assessments (ethologically a more adequate measure of anxiety) in WAG/Rij rats, which was supported by data from both dispersion analysis ($F(1.17) = 13.50$, $p = 0.002$) and post hoc comparison ($t = -2.53$, $p = 0.032$). Administration of imipramine weakened this effect of raclopride (compare WR1B with WR1A and WR2B with WR2A).

In the open field test (Table 2), WAG/Rij rats given placebo (WR1A) showed significantly lower investigative activity (numbers of rearings and excursions to the center of the field) and smaller numbers of grooming reactions than the analogous Wistar group (WS1A). Administration of the antidepressant imipramine, as compared with placebo, improved behavioral characteristics only in WAG/Rij rats, with an increase in investigative activity, i.e., increases in the numbers of rearings ($F(1.17) = 7.22$, $p = 0.015$), excursions to the center of the field ($F(1.17) = 5.91$, $p = 0.026$), and grooming reactions ($F(1.17) = 9.49$, $p = 0.007$). As a result, the difference between WAG/Rij (WR2A) and Wistar rats (WS2A) was insignificant. Administration of imipramine produced no significant changes in behavior in Wistar rats ($F(1.16) \leq 1.24$, $p \geq 0.28$ for all measures) except for a decrease in the number of grooming reactions ($F(1.16) = 5.25$, $p = 0.035$). Administration of raclopride to WAG/Rij rats induced an effect opposite to the effect of imipramine – degradation of behavioral characteristics, with significant reductions in the number of rearings ($F(1.17) = 5.72$, $p = 0.028$) and the number of grooming reactions ($F(1.17) = 4.72$, $p = 0.044$), along with a tendency to a reduction in the number of excursions to the center of the field ($F(1.17) = 3.40$, $p = 0.083$). As a result, WAG/Rij rats given single doses of raclopride after chronic administration of placebo (WR1B) or imipramine (WR2B), like WAG/Rij rats given placebo (WR1A), showed less investigative activity and smaller numbers of grooming reactions than the analogous Wistar groups. In other words, single doses of raclopride both had intrinsic inhibitory effects on behavior (in WR1B) and suppressed the effects of chronic administration of imipramine (in WR2B). On the other hand, administration of raclopride after prior placebo induced more marked degradation of behavioral characteristics (by 55–83%) than after prior imipramine (by 48–53%). Furthermore, in rats given raclopride after placebo (WR1B), measures of behavioral parameters were always less than in rats given raclopride after imipramine (WR2B), in terms of the numbers of rearings ($t = -2.02$, $p = 0.09$), excursions into the center of the field ($t = -3.77$, $p = 0.005$), and grooming reactions ($t = -2.54$, $p = 0.035$). This evidences weakening of the effect of raclopride after

TABLE 2. Behavioral Measures in WAG/Rij and Wistar Rats in the Open Field Test

Behavioral measure	Group of rats							
	WAG/Rij (WR)				Wistar (WS)			
	WR1A (n = 5)	WR1B (n = 5)	WR2A (n = 5)	WR2B (n = 6)	WS1A (n = 5)	WS1B (n = 5)	WS2A (n = 5)	WS2B (n = 5)
Number of squares crossed	61.8 ± 12.01	55.4 ± 11.25	88.6 ± 17.27	71.3 ± 7.80	98.6 ± 18.39	81.6 ± 11.93	91.6 ± 16.87	80.2 ± 16.83
Number of rearings	**6.6 ± 1.40****	**3.0 ± 0.55****+	15.6 ± 4.31#	**7.3 ± 2.08*****	26.0 ± 4.58	21.2 ± 1.77	24.2 ± 5.54	24.8 ± 2.63
Number of excursions to the center of the field	**1.2 ± 0.49***	**0.2 ± 0.19***	3.6 ± 1.54#	1.7 ± 0.33	4.4 ± 1.5	2.0 ± 0.77	2.4 ± 0.51	2.0 ± 0.32
Number of grooming reactions	**2.4 ± 0.68*****	1.0 ± 0.44*+	6.4 ± 1.75#	3.3 ± 0.80	8.6 ± 0.4	6.6 ± 1.69	5.2 ± 0.73##	5.4 ± 0.68
Number of boluses	3.2 ± 0.86	1.6 ± 0.51	2.0 ± 0.95	1.3 ± 0.61	1.2 ± 0.58	0.8 ± 0.49	1.2 ± 0.37	1.0 ± 0.32

Notes. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in WAG/Rij rats as compared with Wistar rats; #$p < 0.05$, ##$p < 0.01$ for group 2A compared with group 1A (effect of imipramine); +$p < 0.05$ in group 1B compared with 1A and group 2B compared with 2A (effect of raclopride). For further details see caption to Table 1.

TABLE 3. Behavioral Measures in WAG/Rij and Wistar Rats in the Sucrose Consumption and Preference Test

Behavioral measures	Group of rats							
	WAG/Rij (WR)				Wistar (WS)			
	WR1A (n = 5)	WR1B (n = 5)	WR2A (n = 5)	WR2B (n = 6)	WS1A (n = 5)	WS1B (n = 5)	WS2A (n = 5)	WS2B (n = 5)
Quantity of sucrose consumed, g	**5.4 ± 0.24***	**1.0 ± 0.32***+++	8.0 ± 0.89#	**3.2 ± 0.83***++	9.8 ± 1.93	7.6 ± 1.86	5.2 ± 0.20	5.4 ± 0.24
Number of approaches to the bowl	7.0 ± 0.71	**2.2 ± 0.66***+++	6.0 ± 0.84	3.8 ± 0.75	7.8 ± 1.69	8.8 ± 1.96	4.4 ± 0.40	5.4 ± 0.24
Quantity of sucrose consumed per approach, g	**0.8 ± 0.06****	0.46 ± 0.04++	1.41 ± 0.21#	0.76 ± 0.11+	1.33 ± 0.13	0.85 ± 0.21	1.22 ± 0.12	1.01 ± 0.06
Sucrose preference (%)	**79.7 ± 3.26****	**68.9 ± 2.61****+	94.8 ± 2.13##	**76.0 ± 2.18****+++	93.0 ± 1.95	91.3 ± 2.89	95.4 ± 1.93	89.65 ± 3.14

Notes. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in WAG/Rij rats compared with Wistar rats; #$p < 0.05$, ##$p < 0.01$ in group A compared with group 1A (effect of imipramine); +$p < 0.05$, ++$p < 0.01$, +++$p < 0.001$ in group 1B compared with 1A and group 2B compared with 2A (effect of raclopride). For further details see caption to Table 1.

prior imipramine. The influence of raclopride in Wistar rats was insignificant for all behavioral measures ($F(1.16) \leq 2.43$, $p \geq 0.14$). Neither imipramine nor raclopride had any significant effect on movement activity (number of squares crossed) in either WAG/Rij or Wistar rats ($F(1.33) \leq 1.67$, $p \geq 0.20$ in all cases).

In the sucrose consumption and preference test (Table 3), the total quantity of sucrose drunk, the quantity of sucrose drunk per approach to the bowl, and the preference (%) for sucrose over water were significantly smaller in placebo-treated WAG/Rij rats (WR1A) than in the corresponding Wistar group (WS1A). Administration of imipramine, as compared with placebo, significantly increased these behavioral measures only in WAG/Rij rats. The differences between WAG/Rij (WR2A) and Wistar (WS2A) rats were therefore insignificant. Treatment of Wistar rats with imipramine produced no significant changes in behavioral measures as compared with placebo ($F(1.16) \geq 0.04$, $p \geq 0.85$), with the exception of a tendency to a reduction in the quantity of sucrose consumed (from 9.8 ± 1.93 to 5.2 ± 0.2, $t = 2.37$, $p = 0.08$). Raclopride had an effect opposite to that of imipramine, producing degradation of behavioral characteristics only in WAG/Rij rats, with decreases in the total quantity of sucrose drunk ($F(1.17) = 47.22$, $p < 0.001$), the quantity of sucrose drunk per approach to the feeder ($F(1.17) = 21.68$, $p < 0.001$), and sucrose preference ($F(1.17) = 33.24$, $p < 0.001$). As a result, WAG/Rij rats given raclopride after placebo (WR1B) or imipramine (WR2B), like WAG/Rij rats given placebo (WR1A), showed lower levels of sucrose consumption and preference

TABLE 4. Behavioral Measures in WAG/Rij and Wistar Rats in the Forced Swimming Test

Behavioral measures	Group of rats							
	WAG/Rij (WR)				Wistar (WS)			
	WR1A (n = 5)	WR1B (n = 5)	WR2A (n = 5)	WR2B (n = 6)	WS1A (n = 5)	WS1B (n = 5)	WS2A (n = 5)	WS2B (n = 5)
Duration of immobility, sec	**223.0 ± 5.61****	**242.0 ± 4.06****+	171.0 ± 14.34#	**221.7 ± 5.87****+	160.0 ± 12.04	175.0 ± 7.58	116.0 ± 14.95#	121.0 ± 11.66
Duration of swimming, sec	**54.0 ± 5.61***	**43.0 ± 4.06***	**84.0 ± 12.29*#**	**58.3 ± 7.15*****	96.0 ± 16.76	76.0 ± 10.88	133.0 ± 13.29	132.0 ± 10.68
Duration of first episode of active swimming, sec	**23.0 ± 2.0***	**17.0 ± 3.74****	45.0 ± 2.24###	**21.5 ± 2.43****+++	44.0 ± 6.20	49.0 ± 5.34	51.0 ± 7.65	45.0 ± 3.87

Notes. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in WAG/Rij rats compared with Wistar rats; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ in group 2A compared with group 1A (effect of imipramine); +$p < 0.05$, ++$p < 0.01$; +++$p < 0.001$ in group 1B compared with 1A and group 2B compared with 2A (effect of raclopride). For further details see caption to Table 1.

than the corresponding Wistar groups. The effect of raclopride on the quantity of sucrose consumed by placebo-treated WAG/Rij rats was greater (an 81.5% reduction) than that in imipramine-treated WAG/Rij rats (a 60% reduction), which is evidence for weakening of the effect of raclopride due to prior administration of imipramine. In Wistar rats, imipramine had no significant effect on any of the behavioral measures studied ($F(1.16) \leq 2.11$, $p \geq 0.17$ in all cases).

In the forced swimming test (Table 4), the duration of immobility in WAG/Rij rats given placebo (WR1A) was greater and the duration of the first episode of active swimming was shorter than in the analogous Wistar group (WS1A). Administration of imipramine to WAG/Rij rats induced a decrease in immobility time ($F(1.17) = 18.98$, $p < 0.001$) and increases in swimming time ($F(1.17) = 8.76$, $p = 0.009$) and the duration of the first episode of active swimming ($F(1.17) = 24.43$, $p < 0.001$) as compared with placebo. As a result, the differences between WAG/Rij (WR2A) and Wistar (WS2A) rats were insignificant. Administration of raclopride had an intrinsic suppressive action on behavior (in WR1B) and prevented the effects of prior administration of imipramine (WR2B). WAG/Rij rats given raclopride after placebo (WR1B) or imipramine (WR2B), like WAG/Rij rats given placebo (WR1A), therefore showed a longer duration of immobility, a shorter duration of swimming, and a shorter first episode of active swimming than seen in the analogous Wistar groups. Furthermore, the duration of immobility in rats given raclopride after placebo (WR1B) was significantly longer ($t = 2.85$, $p = 0.02$) than that in rats given raclopride after imipramine (WR2B), demonstrating weakening of the effect of raclopride by imipramine. The effects of raclopride in Wistar rats were insignificant ($F(1.16) \geq 0.71$, $p \geq 0.41$ for all measures). Administration of imipramine, as compared with placebo, significantly decreased the duration of immobility in Wistar rats ($F(1.16) = 17.09$, $p < 0.001$)

Second experimental series. The second series of experiments addressed the possibility of eliminating depression-like behavioral features in WAG/Rij rats using the dopamine D_2/D_3 receptor agonist parlodel. In the light-dark selection test, parlodel had no significant effect on behavioral measures compared with placebo in either WAG/Rij or Wistar rats: the effects of the "rat strain" factor and the "rat strain" × "parlodel" interaction were insignificant for all behavioral measures. Two-way dispersion analysis showed that $F(1.20) \leq 0.74$, $p \geq 0.40$ for all behavioral measures studied. The effects of the "rat strain" factor on all behavioral measures were also insignificant, with the exception of the number of rearings in the light sector of the chamber ($F(1.20) = 9.60$, $p = 0.006$). Parlodel increased the number of rearings in WAG/Rij rats and decreased this measure in Wistar rats.

In the open field test, parlodel had no significant effect on any of the behavioral measures only in Wistar rats ($F(1.10) \leq 2.16$, $p \geq 0.13$ in all cases), with the exception of a tendency to a reduction in the number of grooming reactions ($F(1.10) = 2.06$, $p = 0.07$). In WAG/Rij rats, parlodel significantly increased the number of rearings (2.83 ± 0.70 with placebo, 7.83 ± 1.42 with parlodel, $F(1.10) = 9.92$, $p < 0.01$) and tended to increase the number of grooming reactions (2.67 ± 0.49 with placebo, 5.17 ± 1.38 with parlodel, $F(1.10) = 2.92$, $p = 0.12$).

In the sucrose consumption and preference test (Fig. 1), parlodel produced significant increases in the total quantity of sucrose consumed only in WAG/Rij rats ($F(1.10) = 9.0$, $p < 0.01$; Fig. 1, *A*), the mean quantity of sucrose consumed per approach to the bowl ($F(1.10) = 36.60$, $p < 0.001$; Fig. 1, *C*), and the preference (%) for sucrose over water ($F(1.10) = 11.90$, $p < 0.01$; Fig. 1, *D*). Administration of parlodel did not alter the number of approaches to the bowl in WAG/Rij rats (Fig. 1, *B*). Parlodel had no significant effect on the behavior of Wistar rats ($F(1.10) \leq 2.66$, $p \geq 0.13$ for all behavioral measures).

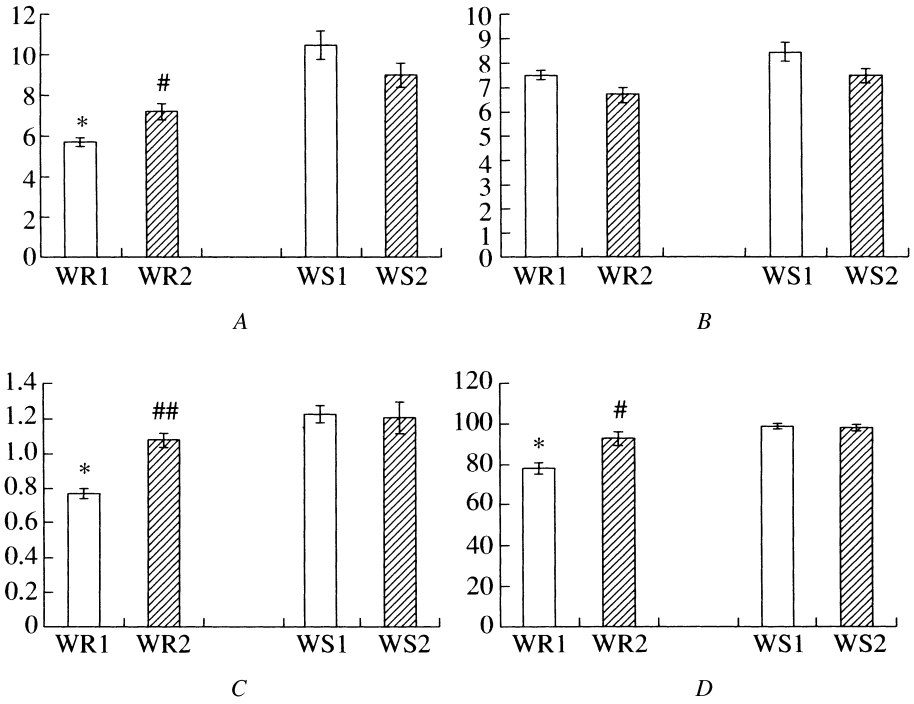

Fig. 1. Effects of parlodel on the behavior of WAG/Rij (n = 12) and Wistar (n = 12) rats in the sucrose consumption (*A*–*C*) and preference (*D*) test. *A*) Total quantity of sucrose consumed, g; *B*) number of approaches to the bowl; *C*) quantity of sucrose consumed per approach to the bowl; *D*) preference (%) for sucrose over water. WAG/Rij rats: WR1 (n = 6) – light columns (placebo), WR2 (n = 6) – shaded columns (parlodel). Wistar rats: WS1 (n = 6) – light columns (placebo), WS2 (n = 6) – shaded columns (parlodel). $^{*}p < 0.01$ for WAG/Rij compared with Wistar rats; $^{\#}p < 0.01$, $^{\#\#}p < 0.001$ for the effect of parlodel compared with placebo (Student's t test).

In the forced swimming test (Fig. 2), administration of parlodel decreased the duration of immobility in WAG/Rij rats ($F(1.10) = 25.70$, $p < 0.001$; Fig. 2, *A*), increased the swimming time ($F(1.10) = 9.80$, $p < 0.01$; Fig. 2, *B*), and increased the duration of the first episode of swimming ($F(1.10) = 12.25$, $p < 0.01$; Fig. 2, *C*), as compared with placebo. In Wistar rats, parlodel significantly decreased the duration of immobility ($F(1.10) = 7.34$, $p < 0.05$; Fig. 2, *A*) and tended to increase the duration of the first episode of active swimming ($F(1.10) = 4.84$, $p = 0.052$; Fig. 2, *C*). As a result of the fact that the effect of parlodel (a reduction in the duration of immobility) was more marked in WAG/Rij rats (by 46.1%) than Wistar rats (by 12%), interstrain differences in this measure after administration of parlodel were insignificant ($t = 1.99$, $p > 0.05$). Two-way dispersion analysis showed that most behavioral measures (the numbers of rearings and grooming reactions, the duration of immobility, the duration of swimming, and sucrose consumption and preference) showed a significant ($F(1.20) \geq 4.94$, $p \leq 0.038$) interaction between the factors "rat strain" × "parlodel," which indicates a relative selectivity for the actions of parlodel on WAG/Rij rats. Analysis of mean behavioral measures provided evidence that parlodel improved most behavioral characteristics only in WAG/Rij rats, producing virtually no changes in behavioral measures in Wistar rats, with the result that the initial interstrain behavioral differences after administration of parlodel were insignificant.

DISCUSSION

Placebo-treated WAG/Rij rats, as compared with the analogous Wistar group, showed differences in behavior such as decreases in investigative activity and the number of grooming responses in the open field test, increased immobility in the forced swimming test, and decreased sucrose consumption and preference. These differences in the behavior of WAG/Rij rats correspond fully to the symptoms of depression-like behavior which we have observed previously in intact rats of this strain [4–6, 24, 25]. Chronic administration of imipramine to WAG/Rij rats increased investigative activity and the number of grooming reactions in the open field test, decreased the duration of immobility and increased the duration of active forms of behavior in the forced swimming test, and increased sucrose consumption and preference. The behavior of WAG/Rij rats after withdrawal of chronic imipramine became indistinguishable

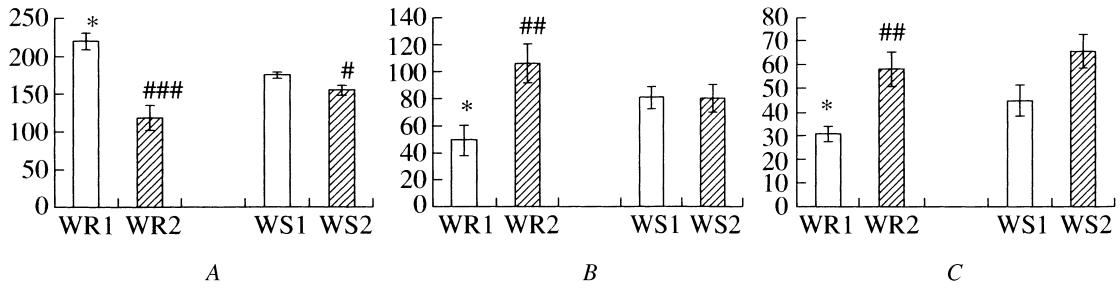

Fig. 2. Effects of parlodel on the behavior of WAG/Rij (n = 12) and Wistar (n = 12) rats in the forced swimming test. *A*) Duration of immobility, sec; *B*) duration of swimming, sec; *C*) duration of first episode of active swimming, sec. $^{*}p < 0.05$ for WAG/Rij compared with Wistar rats; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ for the effect of parlodel compared with placebo (Student's t test). For further details see caption to Fig. 1.

from the behavior of normal rats. In Wistar rats, administration of imipramine only decreased the duration of immobility in the forced swimming test. In other words, chronic imipramine had a therapeutic (antidepressant) effect on the depressive behavior of WAG/Rij rats and had no such effect on the behavior of Wistar rats, with the exception of a reduction in the duration of immobility in the forced swimming test, resulting from the specific characteristics of the Porsolt test [21, 23]. Acute (single-dose) administration of raclopride – a dopamine D_2/D_3 receptor antagonist – suppressed the therapeutic (antidepressant) effect of chronic imipramine. Thus, WAG/Rij rats given raclopride after prior chronic imipramine showed the same depression-like behavior as placebo-treated WAG/Rij rats. The fact that the therapeutic effect of imipramine can be blocked by raclopride has been demonstrated using a model of depression evoked by chronic stress in rats [20]. Blockade of dopamine D_2/D_3 receptors also led to the reappearance of depressive symptoms after they had been eliminated by antidepressant treatment in human patients [30]. Administration of imipramine and raclopride had no significant effect on the level of total movement activity either in WAG/Rij rats or in Wistar rats. The behavioral effects of imipramine and raclopride in WAG/Rij rats cannot therefore be explained by the nonspecific effects of these agents on the animals' total movement activity levels. Raclopride had no significant effect on the behavior of Wistar rats, but increased the symptoms of behavioral depressivity in WAG/Rij rats. The effect of blockade of dopamine receptors in placebo-treated WAG/Rij rats was more marked than in WAG/Rij rats given imipramine. Our studies used raclopride at a low dose (100 μg/kg), which produced no behavioral alterations in Wistar rats. It should be noted that higher doses of raclopride induce a depression-like state and/or catalepsy in normal rats and healthy humans. The increase in the depth of depression-like symptoms induced by low raclopride doses in WAG/Rij rats is therefore evidence that these rats have increased sensitivity to blockade of dopamine D_2/D_3 receptors. The increase in sensitivity to the dopamine receptor antagonist and the decrease induced by chronic imipramine in WAG/Rij rats may be associated, respectively, with a decrease in the functional activity of the mesolimbic system of the brain, as seen in depressive states [12], and the increase induced by antidepressant treatment [12, 22]. The increased sensitivity of D_2-like dopamine receptors in WAG/Rij rats would appear to be a compensatory reaction to hypofunction of the mesolimbic dopaminergic system of the brain. Previous studies have demonstrated an increase in the sensitivity of WAG/Rij rats to the D_1/D_2 receptor antagonist haloperidol, which induces catalepsy [18]. The increased depth of behavioral depressivity symptoms and the suppression of the therapeutic (antidepressant) effect of imipramine induced by blockade of dopamine D_2/D_3 receptors by raclopride provide evidence for the involvement of D_2 dopamine receptors in the pathogenesis of the depression-like behavior seen in WAG/Rij rats and in mediating the therapeutic effect of the antidepressant imipramine. These data obtained in WAG/Rij rats, a new experimental (genetic) model of depression [4, 24], are consistent with data obtained by other authors in another experimental model of depression, i.e., stress-induced depression in rats [20]. Chronic administration of parlodel, a dopamine D_2/D_3 receptor agonist, cured the depression-like behavior of WAG/Rij rats and had no significant effect on the behavior of Wistar rats, with the exception of a reduction in the duration of immobility in the forced swimming test. In other words, the dopamine D_2/D_3 receptor agonist parlodel had a therapeutic (antidepressant) effect comparable to the effect of the tricyclic antidepressant imipramine. Parlodel did not alter the basal levels of movement activity or anxiety in either WAG/Rij or Wistar rats. It should be noted that the therapeutic (antidepressant) effects of imipramine and parlodel were only apparent after withdrawal of chronic administration. During treatment with these agents there was worsening of behavioral characteristics not only in normal Wistar rats, which is consistent with published data [1, 2], but also, and to a greater extent, in depressive WAG/Rij rats (unpublished observations). The positive effect of imipra-

mine appeared earlier (at the end of chronic administration) than that of parlodel (15 days after withdrawal).

Thus, the results obtained here demonstrate the dopamine-dependent nature of depression-like behavior in WAG/Rij rats and point to the involvement of dopamine D_2 receptors in mediating the antidepressant effect of imipramine in genetically determined depression-like behavior in WAG/Rij rats.

CONCLUSIONS

1. WAG/Rij rats given placebo, as compared with the corresponding Wistar group, showed the same depression-like behavior as seen previously in rats of this strain, with decreased investigative behavior and decreased numbers of grooming reactions in the open field test, along with increased immobility in the forced swimming test and decreased consumption of and preference for sucrose solution.

2. Chronic administration of the tricyclic antidepressant imipramine (15 mg/kg for 15 days) had a therapeutic (antidepressant) effect on depression-like behavior in WAG/Rij rats, with increased investigative activity and increased numbers of grooming reactions in the open field test, along with decreased immobility in the forced swimming test and increased consumption of and preference for sucrose solution.

3. Acute (single-dose) administration of raclopride, a dopamine D_2/D_3 receptor antagonist (100 μg/kg 15 min before starting behavioral testing) increased the severity of symptoms of behavioral depressivity and suppressed the antidepressant effect of chronic administration of imipramine in WAG/Rij rats.

4. Chronic administration of parlodel, a dopamine D_2/D_3 receptor agonist (5 mg/kg for 15 days), cured the symptoms of depression-like behavior in WAG/Rij rats.

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