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Are Wistar-Kyoto rats a genetic animal model of depression resistant to antidepressants?

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Abstract

Wistar-Kyoto rats are reported to be very passive in the forced swimming test. In addition, they did not respond to acute administration of either desipramine or 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). In the present experiment, it was studied whether or not they respond to acute and chronic administration of imipramine and the possible relationship to down-regulation of β-adrenoceptors and 5-HT_1 and 5-HT_2 receptors. Sprague-Dawley and Brown–Norway rats were included in the study as it has been previously demonstrated that the two strains respond to acute desipramine and 8-OH-DPAT administration. Whereas acute administration of imipramine (15 mg/kg, three times in a 24 h period) significantly increased struggling and reduced immobility in Sprague-Dawley and Brown Norway rats, Wistar-Kyoto rats failed to respond to the drug. After chronic treatment with imipramine (13 days plus the acute imipramine treatment at the end of the treatment period), the three strains showed a positive response that was always significantly greater than the response to acute administration, but which was much lower in Wistar-Kyoto than in the other two strains. Down-regulation of both β-adrenoceptors and 5-HT_2 receptors was observed 24 h after the forced swimming test in acutely and chronically imipramine-treated rats of the three strains, except that in Sprague-Dawley rats β-adrenoceptors did not change after acute imipramine. No significant decrease in 5-HT_1 binding sites was observed in any strain. Acute imipramine administration caused a similar anorexia in Wistar-Kyoto as in the other strains and at least the same level of down-regulation of β-adrenoceptors and 5-HT_2 receptors. In addition, serum imipramine levels on the day after the last drug administration were higher in Wistar-Kyoto than in the other two strains. All these data suggest that the subsensitivity to imipramine observed in Wistar-Kyoto rats: (i) can not be primarily explained by pharmacokinetic differences, and (ii) does not appear to be related to the monoaminergic systems. Wistar-Kyoto rats might be therefore not only a good animal model of depressive-like (passive) behavior, but also a model of resistance to antidepressants which could be used to investigate the neurobiological basis of such resistance, which is also observed in some depressed patients. © 1997 Elsevier Science B.V.

Keywords: Forced swimming test; Wistar-Kyoto rat; Brown Norway rat; Sprague-Dawley rat; Imipramine; 5-HT_1 receptor; 5-HT_2 receptor; β-Adrenoceptor; Depression

1. Introduction

The forced swimming test was initially described by Porsolt et al. (1977a,b, 1978a) for the screening of antidepressant drugs and was called the behavioral despair test. Several authors interpret the concept coined by Porsolt et al. as similar to learned helplessness (for instance, see O'Neill and Valentino, 1982), and therefore the test has been considered as a putative animal model of depression (Willner, 1984). However, this interpretation was not suggested by Porsolt (1981) and it is probably not appropriate in that exposure to forced swimming does not cause generalized helplessness in rodents, but only modifies the active behavior of animals when further exposed to a similar situation. In the last decade considerable effort has been made to study the actual meaning of the behavior of the animals in the test and two main hypotheses have been elaborated. One considers that the active behavior of ani-

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mals in the forced swimming test is a panic-like reaction (Borsini et al., 1986; Nishimura et al., 1988, 1989), and the other assumes that the test measures the tendency of the animals to adopt active or passive strategies when faced with stressful inescapable situations (Armario et al., 1988; Martí and Armario, 1993). Since the reluctancy to persevere in active behavior when faced with stressful situations might be a component of human depression, the forced swimming test is currently used to relate the immobility of the animals in the test to depressive-like states (Weiss et al., 1981; García-Marquez and Armario, 1987a,b; Paré, 1989a,b). In fact, a positive correlation between the clinical effectiveness of antidepressants and their potency on forced swimming test behavior has been found (Willner, 1984).

Wistar-Kyoto rats have been reported to display low levels of activity in the forced swimming test and high levels of immobility as compared to either normal outbred Sprague-Dawley rats or several inbred strains, including Spontaneously Hypertensive rats (Paré, 1989a,b, 1992; Armario et al., 1995; Lahmame and Armario, 1996; Martí and Armario, 1996). Very recently we have also found that among the five inbred strains of rats studied (Brown Norway, Fisher-344, Lewis, Spontaneously Hypertensive rats and Wistar-Kyoto rats), both Spontaneously Hypertensive and Wistar-Kyoto rats, which are genetically close, failed to respond to acute standard doses of desipramine and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) in terms of enhanced active behavior in the forced swimming test (Lahmame and Armario, 1996). Apparently these strains are rather insensitive to acute antidepressant administration, a finding that has been also reported for outbred rats from different sources (Porsolt et al., 1978b) and for inbred strains of mice in the forced swimming test or similar animal models of depression (Van der Heyden et al., 1987; Trullas et al., 1989).

The lack of appropriate response to acute antidepressant administration is reminiscent of the delayed response of depressed patients to antidepressants in that these drugs have to be administered chronically to be therapeutically effective. Since there is no animal model of resistance to antidepressants, it was considered of interest to study whether rats of a strain that do not respond to acute standard doses of antidepressants do respond to chronic treatment. At present this finding has been reported for Flinders sensitive line rats, which showed both low levels of active behavior in the forced swimming test and a positive response to chronic but not acute imipramine administration (Schiller et al., 1992). However, in this case no other variable was studied in order to demonstrate that the lack of responsiveness to antidepressants affected the forced swimming test only and that pharmacokinetic factors were not involved.

To circumvent these problems, we studied in the present experiment the influence of acute and chronic imipramine administration on forced swimming test behavior. In order to know whether or not the lower behavioral responsiveness to imipramine was linked to some adaptative neurochemical response to antidepressants, we investigated 5-HT_1 and 5-HT_2 receptors and β-adrenoceptors, the two latter types having been reported to be down-regulated in response to a wide range of antidepressants, including imipramine (Banerjee et al., 1977; Peroutka and Snyder, 1980; Charney et al., 1981; Zifa and Fillion, 1992; Burnet et al., 1994). In addition, for appropriate interpretation of the forced swimming test behavior we measured: (i) serum imipramine concentration, which might provide information about drug metabolism and (ii) the anorexia caused by imipramine (Broitman and Donoso, 1978; Blavet and Defeudis, 1982) to include another physiological index of the action of the drug.

2. Materials and methods

2.1. Animals

Male Brown Norway, Wistar-Kyoto and Sprague-Dawley rats approximately 40–45 days old upon their arrival at the laboratory were used. They were obtained from Charles River. The rats were housed (two per cage) in a controlled environment (lights on from 07.30 to 19.30 h, temperature 22°C) for at least 10 days before starting the experiments. Food and water were always provided ad libitum.

2.2. General procedure

The experimental procedures used in this work were previously approved by the ethics committee for animal experimentation of the ''Universitat Autònoma de Barcelona''. Imipramine was dissolved in isotonic saline containing 0.2% agar. Rats from each strain were assigned to one of the three treatments: vehicle, acute or chronic administration of imipramine i.p. The three groups of rats were similarly handled for 15 days and vehicle-treated rats were administered vehicle when the other groups received the drug. Acute imipramine administration (15 mg/kg) occurred three times, twice on the day prior to the test (day 14, at 9.30 and 17.30 h) and once on the 15th day, 1 h before the test. In the case of chronic treatment, imipramine was administered in the morning for 13 days at the dose of 15 mg/kg, two doses on day 14 and the last dose on day 15. Food intake was measured by weighing the amount of food (at 9.00 h) put into the feeders and that remaining in them on the following day and was expressed as g/rat/day, the cage being the experimental unit in the statistical analysis.

2.3. Forced swimming test

In the forced swimming test (Porsolt test), rats were introduced into transparent cylindrical plastic tanks (height

= 40 cm, internal diameter = 19 cm) containing water to a level of 18 cm (25°C). Four identical tanks were used. Two animals, separated by opaque screens, were simultaneously tested, while in the meantime the other two tanks were cleaned and the water was changed for each rat. Only a single exposure to the tank was used because in our experience (Armario et al., 1988, 1991; Lahmame and Armario, 1996) the effect of antidepressants is very reproducible in this condition and the possible interference of memory processes is avoided. During the single exposure to forced swimming (5 min), the behavior of the rat was recorded on videotape. Three types of behavior were distinguished (Armario et al., 1988, 1991; Lahmame and Armario, 1996) and measured with a stopwatch by the same experimenter (A.L.), who was blind to the treatment given to the animals: (i) struggling, which occurred when the rats were diving, jumping or strongly moving all four limbs, the front limbs breaking the surface of the water or scratching the walls, (ii) calm swimming, which occurred when the rats swam around the tank, moving all four limbs, and (iii) immobility, which occurred when the rats remained motionless, keeping their heads out the water.

2.4. Radioligand binding of 5-HT_1 and 5-HT_2 receptors and β-adrenoceptors

Animals were killed by decapitation 24 h after the last imipramine administration. Brains were immediately removed and stored at −80°C until used. Binding assays of 5-HT_1, 5-HT_2 receptors and β-adrenoceptors were carried out in Dr. Pazos's laboratory (Facultad Medicina, Santander, Spain). Cerebral cortexes from 2–3 rats were dissected and pooled, and membranes were prepared as described earlier (Pazos et al., 1984). This resulted in $n = 3–4$ for the statistical analysis of binding data. Briefly, tissues were homogenized in 0.32 M sucrose and the homogenate was centrifuged at $70\,000 \times g$ for 15 min. The pellet was resuspended, incubated at 37°C for 15 min and then centrifuged again. The final pellet was resuspended in assay buffer.

The incubation assay buffer for 5-HT receptors contained 50 mM HCl–Tris (pH 7.7), 4 mM $CaCl_2$, 10 mM pargyline and 0.1% ascorbic acid. Binding assays of 5-HT receptors were performed in a final volume of 1 ml as follows: 750 μl membrane preparation (final dilution 1 g of fresh tissue in 40 ml) were incubated at 37°C for 30 min in the presence of 100 μl of radioligand [^{3}H]5-HT (1–10 nM) to determine 5-HT_1 receptors or [^{3}H]ketanserin (0.5–15 nM) to determine 5-HT_2 receptors. Non-specific binding was determined in the presence of 5-HT for 5-HT_1 receptors and 10^{-6} M methysergide for 5-HT_2 receptors.

To study β-adrenoceptors, membranes were prepared as described by De Paermentier et al. (1989) and binding assays were performed as follows: membranes (750 μl at final dilution 1 g of fresh tissue in 40 ml) were incubated at 27°C for 120 min in the presence of 100 μl of [^{3}H](−)-4-(3-ter-butylamino-2-hydroxypropoxy)-benzimidazol-2-one (CGP-12177) (0.03–2 nM) and 150 μl of buffer. The incubation buffer contained 50 mM HCl–Tris (pH 7.7), 120 mM NaCl, 5 mM KCl and 50 mM $MgCl_2$. Non-specific binding was determined in the presence of 10^{-5} M propranolol.

The incubations were always terminated by rapid filtration (Pazos et al., 1984). The data were analyzed using a Scatchard plot to determine the dissociation constant (K_d) and maximum number of binding sites (B_{max}) by computerized linear regression using EBDA and LIGAND programs (Munson and Rodbard, 1980) included in the RADLIG program (Elsevier-Biosoft) (McPherson, 1985).

2.5. Analysis of serum imipramine

Imipramine concentration was measured by a modification of a previously described method (Mazhard and Binder, 1989; Balíková, 1992), using solid-phase extraction followed by high-performance liquid chromatography (HPLC) with UV detection. The chromatographic system consisted of a Waters 510 pump, a 50 ml fixed loop Rheodyne injector and a model 480 variable-wavelength UV detector.

2.6. Statistical analysis

The statistical significance of the data was initially analyzed by one or two-way analysis of variance (ANOVA). Post-hoc comparisons between two means were made by using the Student's *t*-test and comparisons between more than two means were made by using the Student–Newman–Keuls test ($P < 0.05$). If the variances were not homogeneous, the non-parametric one-way ANOVA (Kruskal–Wallis test) followed by the Mann–Whitney U-test were used.

3. Results

3.1. Effects of both acute and chronic imipramine treatment on forced swimming test behavior

Both initial and final body weight for the 3 strains are indicated in Table 1. Forced swimming test behavior is illustrated in Fig. 1. The two-way ANOVA revealed a significant effect of strain ($F(2,56) = 26.0$, $P < 0.001$) and drug treatment ($F(2,56) = 79.2$, $P < 0.001$) on struggling. The interaction was also significant ($F(4,56) = 4.2$, $P < 0.01$). Post-hoc pairwise comparison showed that Wistar-Kyoto rats displayed lower levels of struggling than the other two strains (Student–Newman–Keuls test). Within each particular strain, the Student–Newman–Keuls tests indicated that: (i) acute imipramine administration increased struggling scores in Brown Norway and Sprague-Dawley rats, but not in Wistar-Kyoto rats; (ii) chronic

Table 1
Initial and final body weight in the three rat strains under the three treatment conditions: vehicle, acute imipramine (IMI) and chronic IMI

	Brown Norway		Sprague-Dawley		Wistar-Kyoto	
	initial	final	initial	final	initial	final
Vehicle	191±3	219±4	315±6	391±8	204±3	237±5
Acute IMI	194±4	220±5	304±4	362±8	208±4	231±5
Chronic IMI	194±3	202±3	319±4	327±3	204±3	195±1

Means and S.E.M. ($n=8$ per group, except for Brown Norway and Sprague-Dawley rats treated chronically with IMI where $n=6$) are presented.

imipramine administration increased struggling in the three strains and the effect was always greater than that observed after acute imipramine.

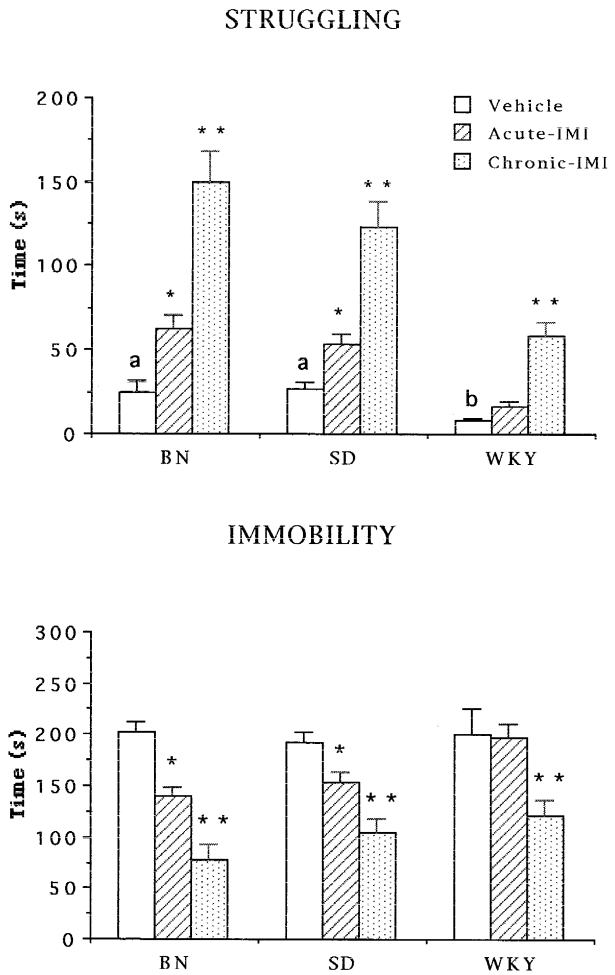

Fig. 1. Effect of acute and chronic imipramine (IMI) treatment on forced swimming test behaviour in three rat strains. Means and S.E.M. ($n=6$–8 per group, except for Brown Norway rats treated chronically with imipramine where $n=5$) are represented. Within vehicle-treated groups, bars labelled with different letters are statistically different (Student–Newman–Keuls test); (*) Statistically significant effect of acute imipramine administration as compared to their respective vehicle-pretreated group; (**) significantly different from both vehicle and acute imipramine treated groups.

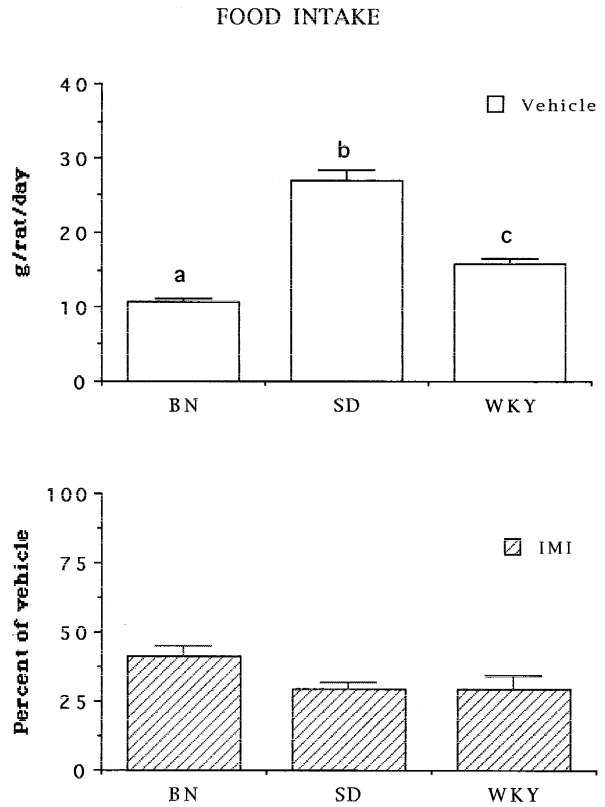

Fig. 2. Effect of acute imipramine (IMI) treatment on food intake in three rat strains. Means and S.E.M. ($n=4$ per group) of absolute food intake (upper panel) or percentage food intake with regard to respective vehicle-treated groups are represented. Within vehicle or imipramine-treated groups, bars labelled with different letters are statistically different (Student–Newman–Keuls test).

The two-way ANOVA revealed a significant effect of strain ($F(2,56)=6.3$, $P<0.01$) and drug treatment ($F(2,56)=45.7$, $P<0.001$) on immobility scores, the interaction being not significant. Post-hoc comparisons showed no significant differences in immobility among the vehicle-treated animals of the different strains. Within each particular strain, the Student–Newman–Keuls test showed that acute imipramine treatment reduced immobility in Brown Norway and Sprague-Dawley rats but not in Wistar-Kyoto rats. The three strains responded to chronic imipramine, the effect being greater than after acute treatment.

3.2. Effects of acute imipramine treatment on food intake

The one-way ANOVA of food intake in vehicle-treated rats revealed a significant effect of strain ($F(2,9)=86.4$, $P<0.0001$). Post-hoc comparisons (Student–Newman–Keuls tests) indicated that Sprague-Dawley rats ate more food than the other strains, Wistar-Kyoto rats showing intermediate levels (Fig. 2). To demonstrate better the effect of acute imipramine treatment on food intake, food intake was represented as a percentage of their appropriate vehicle-treated groups. The Student *t*-test revealed that

acute imipramine caused a profound inhibitory effect on food intake in all strains ($P < 0.001$).

3.3. Effect of acute and chronic imipramine treatment on 5-HT$_1$ and 5-HT$_2$ receptors and β-adrenoceptors

B_{max} and pK_d values for 5-HT$_1$ and 5-HT$_2$ receptors and β-adrenoceptors in Brown Norway, Sprague-Dawley and Wistar-Kyoto rats subjected to acute and chronic imipramine treatments are shown in Fig. 3 and Table 2, respectively. The two-way ANOVA revealed that drug treatment did not alter pK_d values in any strain, but did modify B_{max} in some cases. For 5-HT$_1$ receptors, the two-way ANOVA revealed only a significant effect of strain ($F(2,18) = 4.9$, $P < 0.05$) although a trend toward a decrease in the B_{max} of Brown Norway and Sprague-Dawley (but not of Wistar-Kyoto rats) was detected after both acute and chronic imipramine administration. With regard to 5-HT$_2$ receptors, the two-way ANOVA indicated a statistically significant effect of strain ($F(2,18) = 6.7$, $P < 0.01$) and drug treatment ($F(2,18) = 24.7$, $P < 0.01$), the interaction being not significant. The effect of strain was likely due to a slightly higher number of 5-HT$_2$ receptors in Sprague-Dawley than in Brown Norway or Wistar-Kyoto rats, irrespective of drug treatment. Regarding the influence of drug treatments, post-hoc comparisons revealed that the density of 5-HT$_2$ receptors decreased after acute and chronic imipramine treatment in all strains studied as compared to respective vehicle-treated group. The two-way ANOVA revealed a marginally significant effect of strain ($F(2,18) = 2.7$, $P = 0.09$), but a highly significant effect of imipramine treatment ($F(2,18) = 36.6$, $P < 0.001$) on B_{max} of β-adrenoceptors, the interaction being also significant ($F(4,18) = 3.0$, $P < 0.05$). Post-hoc comparisons showed that the number of β-adrenoceptors was clearly decreased after both acute and chronic imipramine treatment in Brown Norway and Wistar-Kyoto rats. In contrast, down-regulation of these receptors was only significant after chronic imipramine administration in Sprague-Dawley rats.

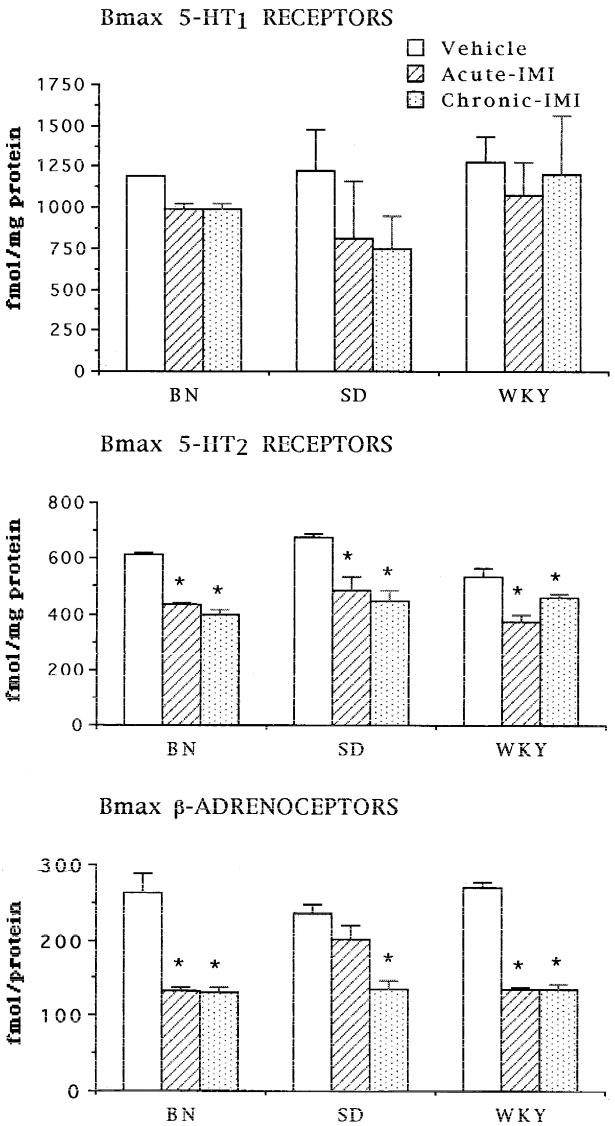

Fig. 3. Effect of acute and chronic imipramine (IMI) treatment on B_{max} of 5-HT$_1$ and 5-HT$_2$ receptors and β-adrenoceptors in brain cortex of three rat strains. Mean and S.E.M. from 3–4 saturation analyses of pooled brain cortexes are presented. B_{max} was derived from Scatchard plots. (*) Statistically significant effect of imipramine administration (acute or chronic) as compared to their respective vehicle-treated group.

Table 2
pK_d of 5-HT$_1$ and 5-HT$_2$ receptors and β-adrenoceptors after acute and chronic administration of imipramine (IMI) to three rat strains

	Brown Norway	Sprague-Dawley	Wistar-Kyoto
5-HT$_1$ receptors			
Vehicle	7.3±0.3	7.5±0.1	7.5±0.6
Acute IMI	8.1±0.1	7.8±0.1	7.6±0.3
Chronic IMI	8.1±0.1	7.6±0.1	7.5±0.3
5-HT$_2$ receptors			
Vehicle	8.5±0.1	8.4±0.1	8.6±0.2
Acute IMI	8.4±0.2	8.5±0.2	8.0±0.5
Chronic IMI	8.4±0.4	8.5±0.3	8.5±0.3
β-adrenoceptors			
Vehicle	9.3±0.1	8.9±0.1	8.8±0.1
Acute IMI	9.5±0.1	8.9±0.0	8.9±0.1
Chronic IMI	9.4±0.6	9.1±0.2	8.8±0.1

Means and S.E.M. from 3–4 saturation analyses (each from a pool of 2–3 pooled brain cortexes) are presented. K_d values were derived from Scatchard plots.

3.4. Serum imipramine concentration after acute and chronic administration

The Kruskal–Wallis test revealed a significant effect of strain on serum imipramine concentration measured 24 h after the last administration in both acutely and chronically treated rats ($P < 0.001$ and $P < 0.01$, respectively, Table

Table 3
Serum imipramine (IMI) concentration (ng/ml) of three rat strains after acute and chronic IMI administration

	Brown Norway	Sprague-Dawley	Wistar-Kyoto
Acute IMI	ND	2983 ± 1287 [a]	7689 ± 1522 [b]
Chronic IMI	2448 ± 278 [a]	1900 ± 800 [a]	7278 ± 804 [b]

Means and S.E.M. ($n = 6$–8 per group, except for Brown Norway rats treated chronically with IMI where $n = 4$) are presented. Within the same treatment, groups labelled with different letters differed statistically (*U*-test). ND = not detectable (< 12 ng/ml).

3). After acute drug administration, post-hoc comparisons revealed higher levels of imipramine in Wistar-Kyoto than in Sprague-Dawley rats, the levels of imipramine being not detectable in Brown Norway rats. After chronic drug administration, serum imipramine levels were higher in Wistar-Kyoto than in Brown Norway ($P < 0.01$) and Sprague-Dawley rats ($P < 0.05$).

4. Discussion

It was found, in accordance with previous results, that Wistar-Kyoto rats showed low levels of active behavior (struggling) in the forced swimming test compared to other outbred or inbred strains (Paré, 1989a,b, 1992; Armario et al., 1995; Lahmame and Armario, 1996; Martí and Armario, 1996). The behavior of the rats in the forced swimming test has been interpreted in two different ways: (i) active behavior (struggling) might be a reflection of a panic-like reaction as a consequence of fear caused by exposure to water (Borsini et al., 1986; Nishimura et al., 1989); (ii) active behavior might be a reflection of the tendency of the animals to adopt active strategies in such an inescapable situation (Armario et al., 1988; Martí and Armario, 1993), and therefore high levels of immobility might be related to a depressive-like state (García-Marquez and Armario, 1987a,b; Weiss et al., 1981). The lack of consistent effect of anxiolytics in the forced swimming test (Porsolt et al., 1977b; Nagatani et al., 1987; Nishimura et al., 1989) and the low levels of activity of emotional as compared to non-emotional rats (Abel, 1991; Overstreet et al., 1992) support the second hypothesis. Therefore it might be initially assumed, on the basis of the present results, that Wistar-Kyoto rats might be prone to adopt passive strategies in apparently inescapable situations and that, at least in this particular aspect, this strain might be a putative genetic animal model of depression.

When the response of different strains of rats to imipramine was studied in the forced swimming test, a positive response to acute imipramine was observed in Sprague-Dawley and Brown Norway but not in Wistar-Kyoto rats. Since we have previously found that this strain also failed to respond to acute standard doses of both 8-OH-DPAT and desipramine (Lahmame and Armario, 1996), it seems likely that Wistar-Kyoto rats are unresponsive to most antidepressants. Since the clinical efficacy of antidepressant drugs is achieved after several weeks of treatment, we also studied the response of the animals to chronic imipramine treatment. A greater response to chronic as compared to acute imipramine was observed in all strains. This enhanced response after chronic antidepressant administration is consistent with previously published data in outbred rats (Kitada et al., 1981) and cannot apparently be explained in terms of pharmacokinetic changes caused by the chronic drug administration (Poncelet et al., 1986; Mancinelli et al., 1987). Such a finding could be related to the progressive time course of clinical improvement of depressive symptoms during antidepressant therapy. Although Wistar-Kyoto rats showed a positive response to chronically administered imipramine in terms of increased activity in the forced swimming test, they responded far less than Brown Norway and Sprague-Dawley rats, suggesting that Wistar-Kyoto rats might be a good model for partial resistance to antidepressant administration.

In the present work some particular adaptative neurochemical responses were studied mainly to rule out the influence of inter-strain differences in the biological efficacy of the drug. Nevertheless, the data could also be relevant in explaining the neurochemical mechanisms involved in the differential responsiveness to antidepressant (imipramine) treatment of these three strains. Acute and chronic imipramine administration caused a similar down-regulation of cortical β-adrenoceptors and 5-HT_2 receptors in the three strains, excepting that acute imipramine had no effect on β-adrenoceptors in Sprague-Dawley rats. Although it is generally assumed that down-regulation of β-adrenoceptors and 5-HT_2 receptors is observed after chronic but not acute administration of antidepressants (Charney et al., 1981), acute imipramine treatment has been found to cause down-regulation of β-adrenoceptors and 5-HT_2 receptors in Fischer rats (Paul et al., 1990), suggesting that some strains are particularly sensitive to down-regulation. This strain-dependent effect is confirmed by our present results. Both acute and chronic imipramine treatments failed to affect 5-HT_1 receptors, although a trend toward a decrease was observed in Brown Norway and Sprague-Dawley rats.

Down-regulation of β-adrenoceptors has been observed after acute imipramine administration to Sprague-Dawley rats only when the drug treatment was followed by exposure to the forced swimming test, as it was in the present study (Duncan et al., 1985; Paul et al., 1990). In addition, chronic stress has been reported to down-regulate β-adrenoceptors (Stone, 1988). Therefore it is possible that the changes in receptors observed in the present study might be the result of an interaction between the mild chronic stress associated with daily injection, exposure to the forced swimming test and drug treatment. In the present experiment groups of rats that received each saline or drug treatment but which were not exposed to the forced swim-

ming test were not included because the simultaneous comparison of three strains makes the study extremely difficult. However, we think this drawback does not alter the main conclusion that Wistar-Kyoto rats responded very well in terms of monoaminergic receptor down-regulation to the experimental procedures for the following reasons: (i) the possible effect of stress associated with daily injection and exposure to the forced swimming test was also present in vehicle-treated animals, (ii) down regulation of β-adrenoceptors after chronic stress usually disappears when the animals are killed 24 h after the last exposure to stress (Stone, 1988) as is the case in the present study, and (iii) chronic stress results in up-regulation rather than down-regulation of 5-HT_2 receptors (Takao et al., 1995). Therefore it is unlikely that an altered monoaminergic response is the main reason for the weak response of Wistar-Kyoto rats to antidepressants. Incidentally, the present results also suggest that cortical 5-HT_1 and 5-HT_2 receptors and β-adrenoceptors might not be directly involved in the effect of antidepressants (imipramine). Since thyroid hormones have been used in treatment of refractory depression (Thase and Rush, 1995), we measured serum thyroxine levels in these strains before and after chronic imipramine administration and no differences were found (unpublished data). The exact neurochemical mechanisms involved in the down-regulation of 5-HT_2 receptors and β-adrenoceptors caused by most antidepressants remain unclear. Regardless of the exact mechanism of action of the drugs, the finding that both β-adrenoceptors and 5-HT receptors were altered is not surprising, considering that there is evidence for a functional link between serotonergic and noradrenergic neuronal systems (Gravel and De Montigny, 1987; Pryor and Sulser, 1991).

The possibility that the blunted responsiveness of Wistar-Kyoto rats to imipramine could have been due to pharmacokinetic differences might be considered. However, again several of our findings argue against this hypothesis: (i) the lack of responsiveness to acute administration has been observed with three different antidepressants (imipramine in the present experiment, desipramine and 8-OH-DPAT in Lahmame and Armario, 1996) with very different pharmacokinetic profiles; (ii) in a previous study (Lahmame and Armario, 1996), 4 different doses of desipramine and 2 doses of 8-OH-DPAT were tested and confirmed the blunted response of WKY rats; (iii) the anorexia caused by imipramine was similar in the three strains; (iv) down-regulation of 5-HT_2 receptors and β-adrenoceptors was evident in Wistar-Kyoto rats even after acute imipramine treatment; (v) Wistar-Kyoto rats showed a higher serum imipramine concentration than the other strains after both acute and chronic administration, when measured 24 h after the last drug administration. Although desmethylimipramine, the main imipramine metabolite, could not be measured, both compounds have similar effects on behavior in the forced swimming test and it is therefore unlikely that an altered desmethylimipramine/imipramine ratio could have contributed to the inter-strains differences. If the defect in the responsiveness of Wistar-Kyoto rats does not appear to be primarily due to pharmacokinetic differences, this might be related to specific alterations in neurotransmitters or areas involved in the control of behavior in the forced swimming test. This might explain both the low levels of activity in drug-free animals and the weak, if any, response to antidepressants. On the basis of the present results we have no evidence that altered cortical monoaminergic activity is the cause of the abnormal behavior of Wistar-Kyoto rats in the forced swimming test or their low responsiveness to antidepressants. However, other areas (i.e. the habenula, Thornton et al., 1990) have been implicated in the control of forced swimming behavior or in the action of antidepressants (i.e. the amygdala, Gorka et al., 1979; Araki et al., 1985; Duncan et al., 1986), and neurotransmitters other than monoamines should be studied as the origin of the resistance of Wistar-Kyoto rats to antidepressants.

In summary, the present data confirm that Wistar-Kyoto rats are very passive in the forced swimming test. In addition it was observed that Wistar-Kyoto responded with increased activity in the test only after chronic treatment with a standard dose of the antidepressant imipramine and even in this case the effect was much lower than in Brown Norway and Sprague-Dawley rats, whose response to imipramine was strong and fast (positive response to acute drug administration). The defective response of Wistar-Kyoto rats to imipramine in the forced swimming test appears not to be related to 5-HT_2 receptors or β-adrenoceptors since these receptors were down-regulated at least to the same degree as in the other strains. Assuming that low levels of activity in the forced swimming test measures some particular aspects of depression-like behavior (reluctancy to maintain effort in apparently inescapable stressful situations), Wistar-Kyoto rats might be a good genetic animal model of depression showing resistance to antidepressants, as is observed in some populations of depressed patients.

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