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Antiaggresive and Anxiolytic Effects of Gepirone in Mice, and Their Attenuation by WAY 100635

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MENDOZA, D. L., H. A. BRAVO AND H. H. SWANSON. *Antiaggressive and anxiolytic effects of Gepirone in mice, and their attenuation by WAY 100635*. PHARMACOL BIOCHEM BEHAV **62**(3) 499–509, 1999.—The purpose of the investigation was to ascertain whether (a) the antiaggressive effects of the 5-HT_{1A} partial agonist, Gepirone, could be mediated via its anxiolytic action; (b) the selective 5-HT_{1A} antagonist, WAY 100635, reversed these effects, and (c) the modulation of "stress hyperthermia" could be attributed to direct effects of the drugs. Isolated male mice were treated with WAY 100635 (0, 1.5, 2.5, and 5 mg/kg) given 15 min prior to Gepirone (0, 2.5, 5, and 7.5 mg/kg). Rectal temperature was taken before the first injection and again prior to the behavioral tests. In the first session only, subjects were tested for anxiety on the elevated plus-maze before the resident–intruder test. Gepirone reduced aggression in a dose-dependent manner. This effect was counteracted by all doses of WAY 100635. On the elevated plus maze, Gepirone increased open-arm entries and duration and reduced risk assessment. The largest dose of WAY 100635 had a mild direct anxiolytic action, but all doses reduced the anxiolytic action of the largest dose of Gepirone. Body temperature was decreased dose dependently by Gepirone, an effect prevented by WAY 100635. The results justify attributing the involvement of the 5-HT_{1A} receptors in the modulation of aggression and anxiety. © 1999 Elsevier Science Inc.

Gepirone WAY 100635 Territorial aggression Anxiety Mice

IN a previous article we suggested that the partial 5-HT_{1A} agonist, Gepirone, suppressed aggression by a resident mouse by decreasing anxiety caused by invasion of the territory by an intruder. Furthermore, the 5-HT_{1A} antagonist, WAY 100135, counteracted the antiaggressive action of Gepirone (29). The purpose of the present investigation is to examine whether the regime of Gepirone previously used does indeed have a direct anxiolytic action by using the elevated plus-maze as measure of anxiety before subjecting the animals to a resident–intruder test. Second, because some dose-related actions of WAY 100135 were ambiguous, various doses of a more selective 5-HT_{1A} antagonist of the same class, WAY 100635, were administered to see whether the antiaggressive, anxiolytic, and hypothermic actions of Gepirone could be reversed.

Ethologically, it is well established that anxiety and aggression are related. The specific relationship between anxiety and aggression depends on the situation and its adaptive value. The defense of one's territory is obviously advantageous, and the sudden appearance of an intruder in his cage will be perceived as a threat by the resident. Because isolation is known to engender anxiety (23) as well as irritability (51,52), the previously isolated resident will be highly motivated to attack the intruder (27,40). But if the overall level of anxiety is decreased by drug treatment, his predilection to fight may also be reduced.

As Gepirone is generally regarded as anxiolytic, both clinically (12,35,38) and under several experimental conditions (48–50,53), it seemed reasonable to assume that the decreased level of territorial aggression seen in our previous study could be due to its anxiolytic action. The increase in attending and scanning and decreased grooming supports this suggestion, but a direct measure of anxiety seemed appropriate. The elevated plus-maze is a well-established paradigm for the study of anxiety. Using this paradigm, Rodgers examined the effects of the classical 5-HT_{1A} agonists, and found that anxiolytic effects were evident at doses low enough not to have debilitating effects on activity (22,39).

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In a more recent study, File et al. (16) showed that in rats direct administration of 8-OH-DPAT into the median raphe nucleus produced a decrease in anxiety in the social interaction test as well as in the plus-maze. This effect was suppressed by simultaneous intracerebral injection of WAY 100635. Conversely, injection of 8-OH-DPAT into the dorsal hippocampus increased anxiety, an effect antagonized by WAY100135 (administered SC). The results provided evidence that stimulation of presynaptic receptors results in anxiolysis, whereas the postsynaptic receptors are anxiogenic. Although the overall action of 5-HT_{1A} agonists is to reduce 5-HT production in the dorsal hippocampus, the opposition of pre- and postsynaptic receptors may explain the relatively weak anxiolytic profile seen with systemic administration of 5-HT_{1A} agonists (16,22,28).

The replacement in the present experiment of the newer compound WAY 100635 for the one used in our previous study (WAY 100135) was motivated by reports of greater potency and selectivity (1,18). Autoradiographic, binding, and electrophysiological assays have demonstrated a high and selective affinity for 1A serotonin receptors and strong antagonistic action both pre- and postsynaptically (11,17,19). Multiple effects of the classical full agonist 8-OH-DPAT, as well as of various partial agonists, both at the cellular and behavior level, have been counteracted by WAY 100635 (4,5,17,19,24, 31,54). With particular reference to the present study, attenuation of antiaggressive activity of 5-HT_{1A} agonists (32,43) as well as reversal of their anxiolytic effects (10,16,37,48,54) have been reported. Although in most of the reviewed work WAY 100635 has been devoid of effects of its own (1,4,8,19,32), recently some direct anxiolytic effects of WAY 100635 have been mentioned (6,17). The design of the present study permitted an evaluation of any such direct action of WAY 100635 on anxiety. In spite of reports that lower doses of WAY 100635 were as effective as the less selective WAY 100135 on certain processes (9,13,18,19,33, 46), we felt that the better insight on the mode of action of the two WAY compounds on the modulation of territorial aggression would be obtained by using comparable doses and timing of administration for WAY 100635 in the present work as we used for WAY 100135 in a previous experiment (29). Moreover, the interval between drug injection and behavioral testing could be justified by the report that, using autoradiography, optimal labeling of 5-HT_{1A} sites in the brain was obtained 1 h after IV injections of [^{3}H]WAY 100635 (28).

Finally, the suppression of stress-induced hyperthermia by Gepirone and its reversal by WAY 100135, observed in our earlier experiment by comparing pre- and posttest rectal temperature, was further studied by investigating the direct action of the drugs before subjecting the animals to the resident–intruder test, this time using the newer compound, WAY 100635.

METHOD

Experimental protocol was approved by University Bioethical Commision as being in compliance with the European Comunities Council Directive of 24 November 1986 (86/609/EEC).

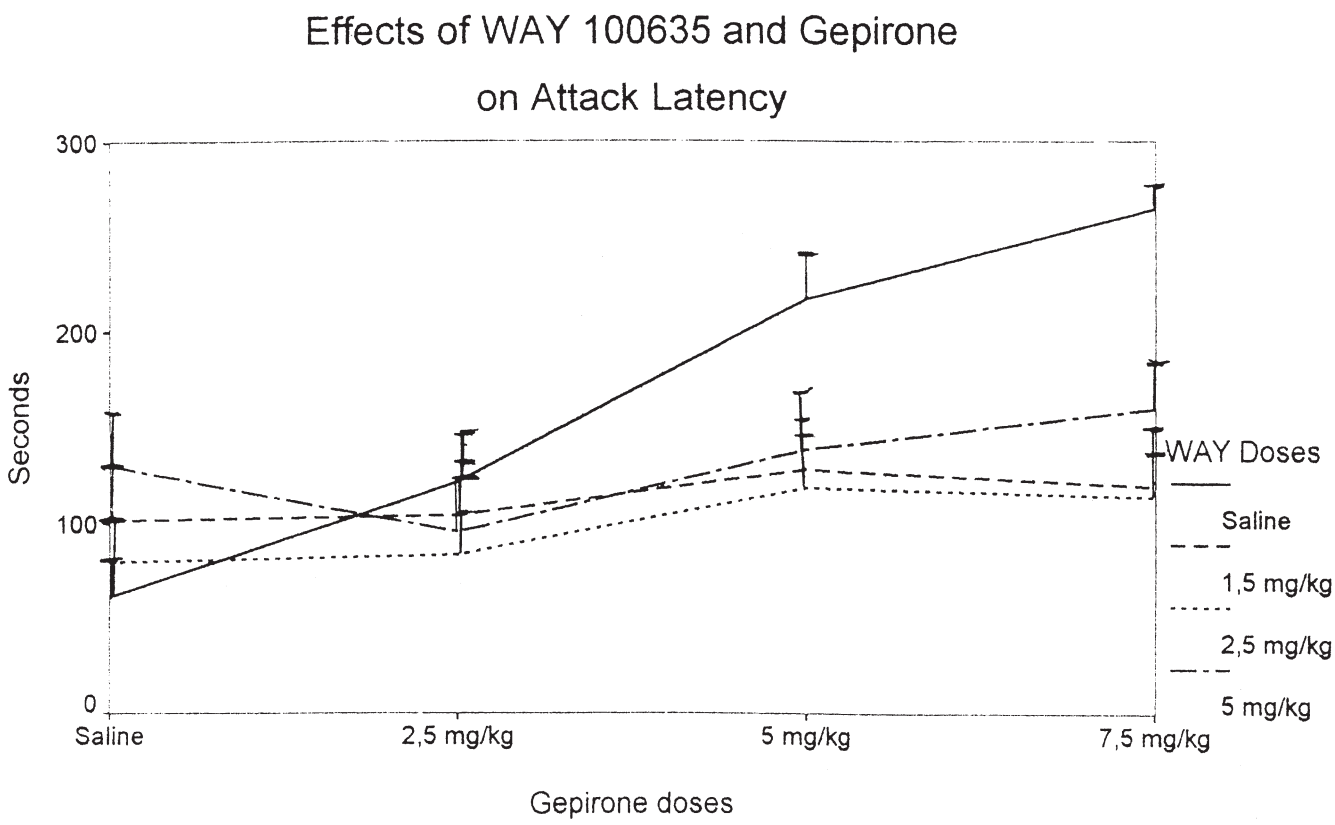

FIG. 1. Effects of Gepirone and (+)WAY 100635 on attack latency. Mean latencies and SEM of each treatment group. Each line represents the effects of 0, 2.5, 5, and 7.5 mg/kg of Gepirone given to the same subjects (within-subject factor). The three different lines represent the additional effect of WAY pretreatment at the 0, ,1.5, 2.5, and 5.0 mg/kg level (between-subjects factor). The total duration of the test was 300 s.

Subjects

All subjects were adult male mice of the BALB/C strain weighing 35 to 45 g. The heaviest animals were used as RESIDENTS. These were housed in individual Plexiglas cages (size 16 × 10 × 6 cm) for 2–3 weeks before the beginning of each experiment. The remaining animals (INTRUDERS) weighed at least 10% less than residents and were placed in group cages of five animals per cage. All animals received food and water ad lib, and were kept under 12-h reversed-light conditions. Tests were carried out during the dark (active) period.

Drugs

Gepirone (Bristol-Myers Squibb Pharmaceuticals Ltd.) and (+) WAY 100635 ({*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide}) (Wyeth Research Ltd.) were administered IP diluted in physiological saline solution (4 ml/kg). Controls received a similar volume of saline. All treatments were given only to the resident. Gepirone was administered 15 min after the pretreatment with WAY 100635, and testing began 30 min after the second injection.

Apparatus

The elevated plus-maze was based on the model described by Rodgers (41). Two open arms (30 × 5 × 0.25 cm) and two enclosed arms (30 × 5× 15 cm) extended from a central platform (5 × 5 cm), making the shape of a plus sign. The apparatus was elevated 45 cm from the floor. The maze floor was made of block board covered by formica and grip was provided on the open arms by a slight lip (0.25 cm). The tests were performed under normal room illumination, and a video camera was suspended over the apparatus for recording activity.

Test Procedures

Elevated plus-maze. The anxiety test was performed only once per animal, on its first experimental session. Thirty minutes after the second injection, body temperature was measured and, immediately afterwards, the mouse was placed in the elevated plus maze for 5 min. The session was recorded on video and the behavior of the mouse scored according to the criteria of Rodgers (41).

Traditional measures as well as recently developed ethological measures were scored. Open- and closed-arm entries and risk assessment behaviors: return, protected, and unprotected head dips and stretches, were scored in frequencies. Measures of duration were: time spent in the central platform, open, and closed arms. The following percentages were calculated: percentage of open-arm entries, open-arm duration, and protected stretches and head dips. Percentages were referred to their respective totals.

Resident–intruder test. Immediately after the anxiety test, the first resident–intruder test was carried out. On subsequent

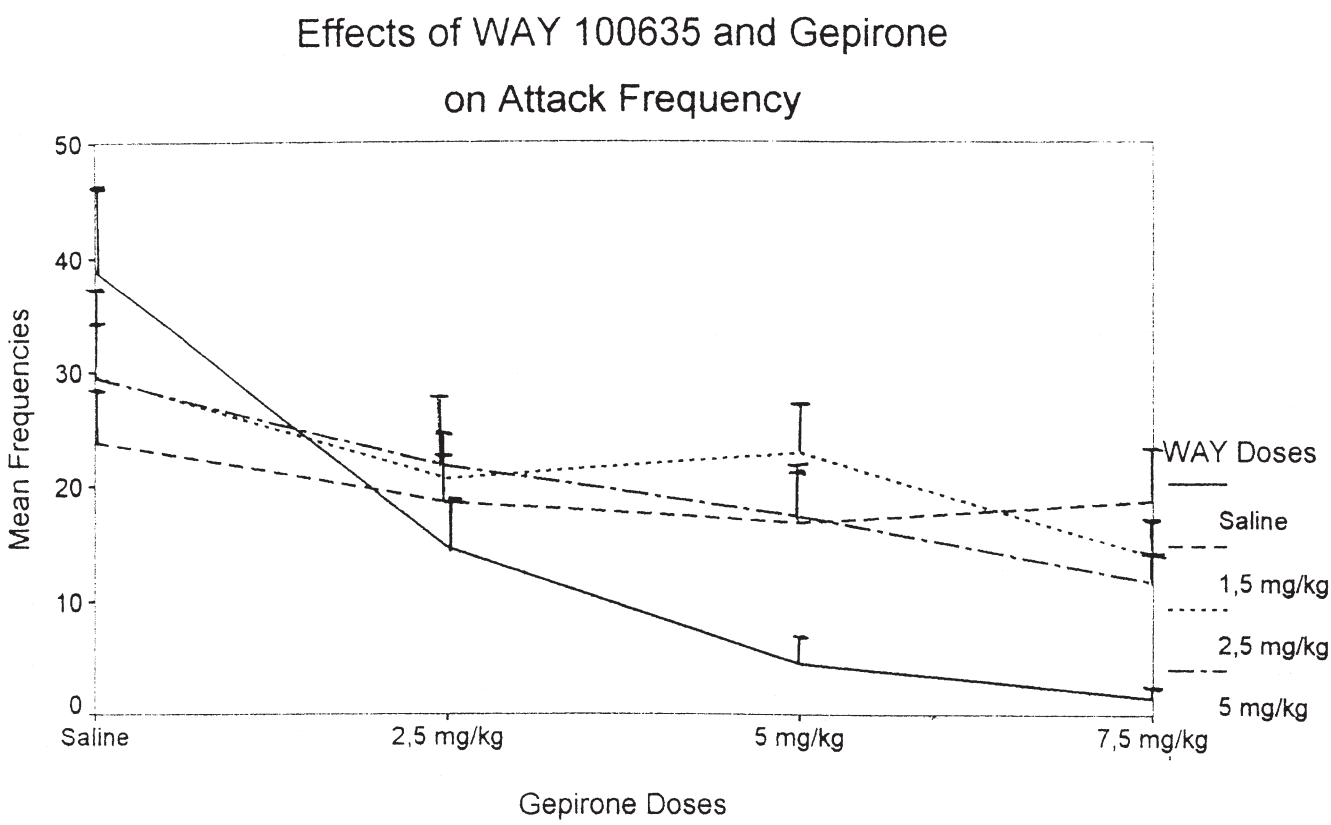

FIG. 2. Effects of (+) WAY 100635 and Gepirone on attack frequency. Mean frequencies and SEM of each treatment group. Each line represents the effects of 0, 2.5, 5, and 7.5 mg/kg of Gepirone given to the same subjects (within-subject factor). The three different lines represent the additional effect of WAY pretreatment at the 0, 1.5, 2.5, and 5.0 mg/kg level (between-subjects factor).

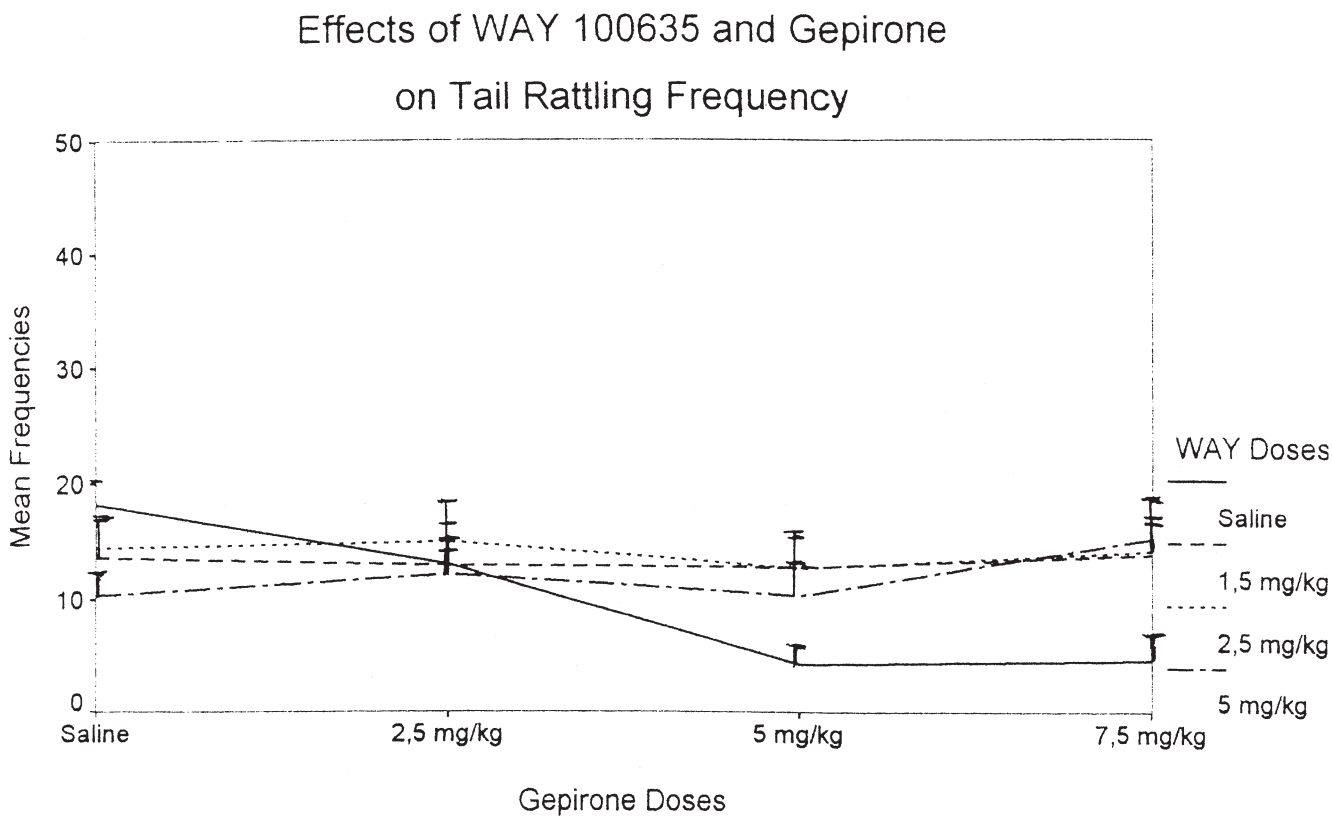

FIG. 3. Effects of (+)WAY 100635 and Gepirone on tail-rattling frequency. Mean frequencies and SEM of each treatment group. Each line represents the effects of 0, 2.5, 5, and 7.5 mg/kg of Gepirone given to the same subjects (within-subject factor). The three different lines represent the additional effect of WAY pretreatment at the 0, 1.5, 2.5, and 5.0 mg/kg level (between-subjects factor).

tests body temperature was measured before injection of the first drug and again just before the intruder was placed in the resident's cage. Aggression tests began on introduction of the intruder. These were recorded on video for exactly 5 min.

Behavior

Only the resident's behavior was scored according to the criteria of Grant and Mackintosh (21). Although defensive behaviors such as upright and sideways defensive postures, escape attempts, and flight were occasionally exhibited by the resident, these were not considered, as they depended on the aggressive attacks by the intruder. The frequencies of these behaviors were very low.

Aggressive behavior. Latency for the first attack as well as the frequencies of attack, chase, and tail rattle were scored.

Nonaggressive behaviors. Social sniff (any part of the body of the intruder), self-groom, exploratory sniff (subject sniffs

TABLE 1
NONAGGRESSIVE BEHAVIOUR OF RESIDENTS

Gepirone Doses (WAY = 0)	Gep = 0	Gep = 2,5	Gep = 5	Gep = 7,5	ANOVA	
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	$F(3,132)$	p
Exploratory sniffing	30.3 ± 3.5	33.0 ± 3.6	37.4 ± 4.2	41.8 ± 2.2	3.63	.015
Attending	3.3 ± 1.9	11.3 ± 6.0	31.2 ± 7.7	37.5 ± 14.4	5.28	.002
Social sniffing	8.1 ± 2.11	18.5 ± 4.2	8.0 ± 2.8	15.0 ± 4.8	2.77	.044
WAY Doses (Gepirone = 0)	WAY = 0	WAY = 1,5	WAY = 2,5	WAY = 5	ANOVA	
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	$F(3,44)$	p
Grooming	5.1 ± 8.2	4.7 ± 1.1	9.5 ± 2.2	8.4 ± 2.3	5.13	.004

Mean and standard error (SE) of behaviours that showed significant effects of Gepirone alone (exploratory and social sniffing, and attending) or WAY alone (grooming).

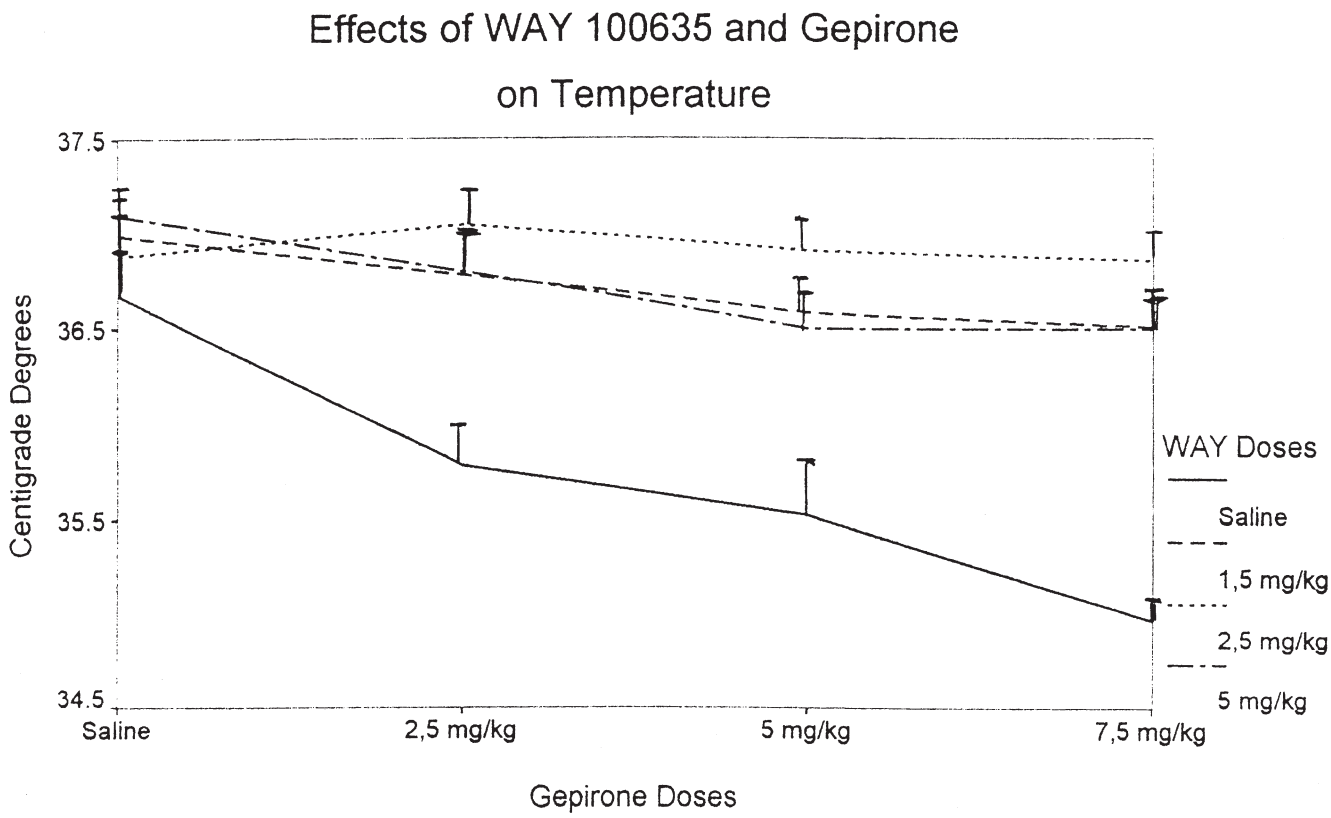

FIG. 4. Effects of (+)WAY 100635 and Gepirone on rectal temperature. The graph represents the mean and SEM of temperatures (°C) taken before the test. Each line represents the effects of 0, 2.5, 5, and 7.5 mg/kg of Gepirone given to the same subjects (within-subject factor). The three different lines represent the additional effect of WAY pretreatment at the 0, 1.5, 2.5, and 5.0 mg/kg level (between-subjects factor).

cage or air) were scored as frequencies, whereas sitting attending (the subject is sitting still while scanning the surroundings or intruder) was scored as duration in seconds.

Scoring

The main investigator marked the scoring sheet each time a specific behavior occurred. Some of the measures were taken twice, and correlation between them were calculated, and found to be above 0.80. The mean of both scores were used for statistical analysis.

Body Temperature

Rectal temperature was taken twice per subject in each session: before the first injection and before any experimental test. The mouse was restrained manually, and a normal fever thermometer was inserted into the anus for 2 min.

Statistics

An SPSS computer program was used for statistical analysis. Kolmogorov-Smirnof test showed that data followed a normal distribution, allowing the use of paramentrical tests. The attack latency, frequency, or duration of aggressive behavioral elements were analyzed by repeated-measures ANOVA with one within-subject factor (Gepirone) and one between-subject factor (WAY), while frequency, duration, and percentage calculations of anxiety measures were analyzed by a factorial analysis, with two independent factors, WAY and Gepirone. *t*-Test for related and independent samples, respectively, were calculated where appropriate.

Experimental Design

A two-factor (4 × 4) design was used: Gepirone had four dose levels (1, 2.5, 5, and 10 mg/kg), and the pretreatment with WAY had four (0, 1.5, 2.5, and 5 mg/kg). There was a total of 16 combinations of treatments. Forty-eight residents were used (three residents per cell) and in the case of the aggression tests, the factorial design was repeated four times. Anxiety tests were performed only once per subject, being the first experimental procedure to which each animal was exposed. Therefore, the total number of tests was 240 (192 aggression tests plus 48 anxiety tests). In each experimental session, each animal was injected twice: in the first place with WAY, and 15 min afterwards with Gepirone. The test started 30 min after the second injection. In the first test session, because an elevated plus-maze test was interpolated, the resident–intruder tests began 50 min after the first injection instead of 45 min. By means of the repetition, every subject always received the same preliminary dose of WAY (between-subjects factor), followed by each of the four Gepirone treatments in a different order (within-subjects factor). To control for the possible effect of a repeated aggressive experience, four "special controls," injected twice with saline before each session, were tested on four consecutive occasions, together with the experimental subjects.

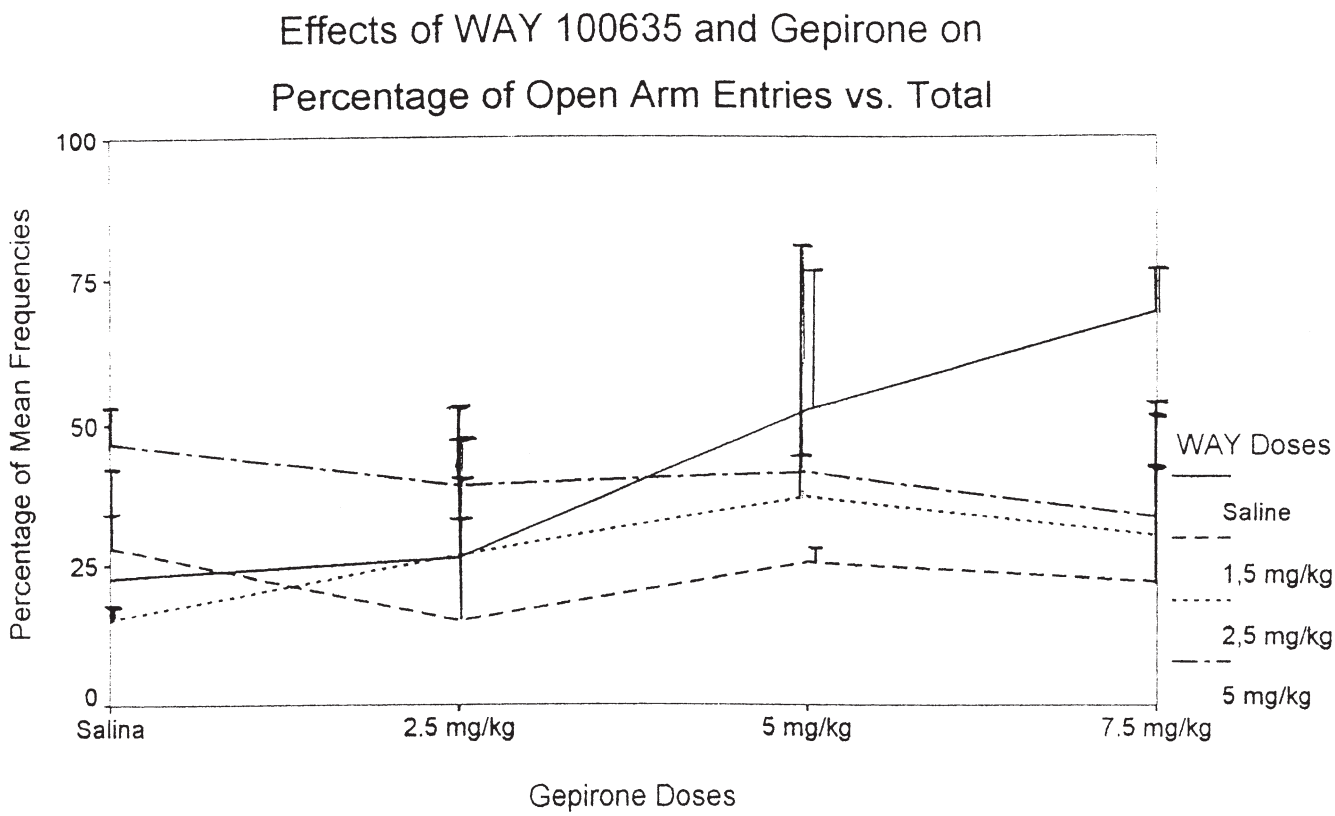

FIG. 5. Effects of (+)WAY 100635 and Gepirone on open-arm entries. Open-arm entries expressed as the percentage of total entries [open-arm entries ÷ (open- + closed-arm entries) × 100]. Mean and SEM of each treatment group. Each line represents the effects of 0, 2.5, 5, and 7.5 mg/kg of Gepirone and the three different lines represent the additional effect of WAY pretreatment at the 0, 1.5, 2.5, and 5.0 mg/kg level.

RESULTS

Resident–Intruder Test

Aggressive behavior (Figs. 1, 2, and 3). Gepirone decreased all measures of aggression in a dose-dependent manner [attack latency, $F(3, 132) = 8.99$, $p < 0.001$; attack frequency, $F(3, 132) = 15.00$, $p < 0.001$; tail rattling, $F(3, 132) = 2.51$, $p = 0.06$]. There was no ceiling effect, as even with the largest dose (7.5 mg/kg) some attacks did occur. WAY had absolutely no effect on aggression insofar as none of the doses of WAY produced levels of aggression different from controls. All doses of WAY reduced the action of Gepirone to the level of the controls, there being no difference in the efficacy of any of the doses. The interaction between Gepirone and WAY was highly significant on all measures [attack latency, $F(9, 132) = 2.76$, $p < 0.01$; attack frequency, $F(9, 132) = 2.37$, $p < 0.05$; tail rattling, $F(9, 132) = 2.58$, $p < 0.01$].

Nonaggressive behavior (Table 1). Exploratory sniffing was increased by Gepirone in a dose-dependent manner. Although there appeared to be a significant overall effect of Gepirone on social sniffing, there was no consistent pattern attributable to the various doses. Attending was increased by Gepirone, particularly with the larger doses. Grooming was not affected. WAY had no direct effects, except on grooming, which was increased. There were no significant interactions between Gepirone and WAY on any measures. These results indicate that the decreased aggression with Gepirone could not be attributed to sedation. There was no observable evidence of motor impairment. The increase in attending suggests more attention being paid to the environment, including the intruder, before initiation of an attack.

Special controls. The special controls showed no difference in any of the measures of aggressive or nonaggressive behavior between the four tests, indicating that fighting experience had no influence. The order of testing of animals receiving various drug regimens should, therefore, not confound the results.

Body temperature (Fig. 4). Gepirone had a marked direct dose-dependent hypothermic action, $F(3, 126) = 9.21$, $p < 0.001$, whereas WAY increased body temperature, $F(3, 42) = 15.94$, $p < 0.001$. Furthermore, WAY completely prevented the temperature rise induced by Gepirone. This effect was not dose dependent [interaction, $F(9, 126) = 2.29$, $p < 0.05$].

Elevated Plus-Maze

Open- and closed-arm entries (Fig. 5). There was no difference between groups in total entries, which is a good measure of activity. An increase in the percent of entries into open arms vs. total entries (open plus closed arms) is the most economical way of showing a decrease in anxiety. Although the overall effect of Gepirone did not reach significance because of the large variability, the graph shows that the higher doses of Gepirone increased open arm entries. Indeed, follow-up analysis revealed a significant effect at 7.5 mg/kg [Sal vs. 7.5 mg Gep, $t(4) = 5.48$, $p < 0.01$]. Surprisingly, WAY alone also increased open-arm entries, albeit only with the largest dose [Sal vs. 5 mg WAY, $t(4) = 2.89$, $p < 0.05$]. Although the overall interaction factor did not reach significance, the graph

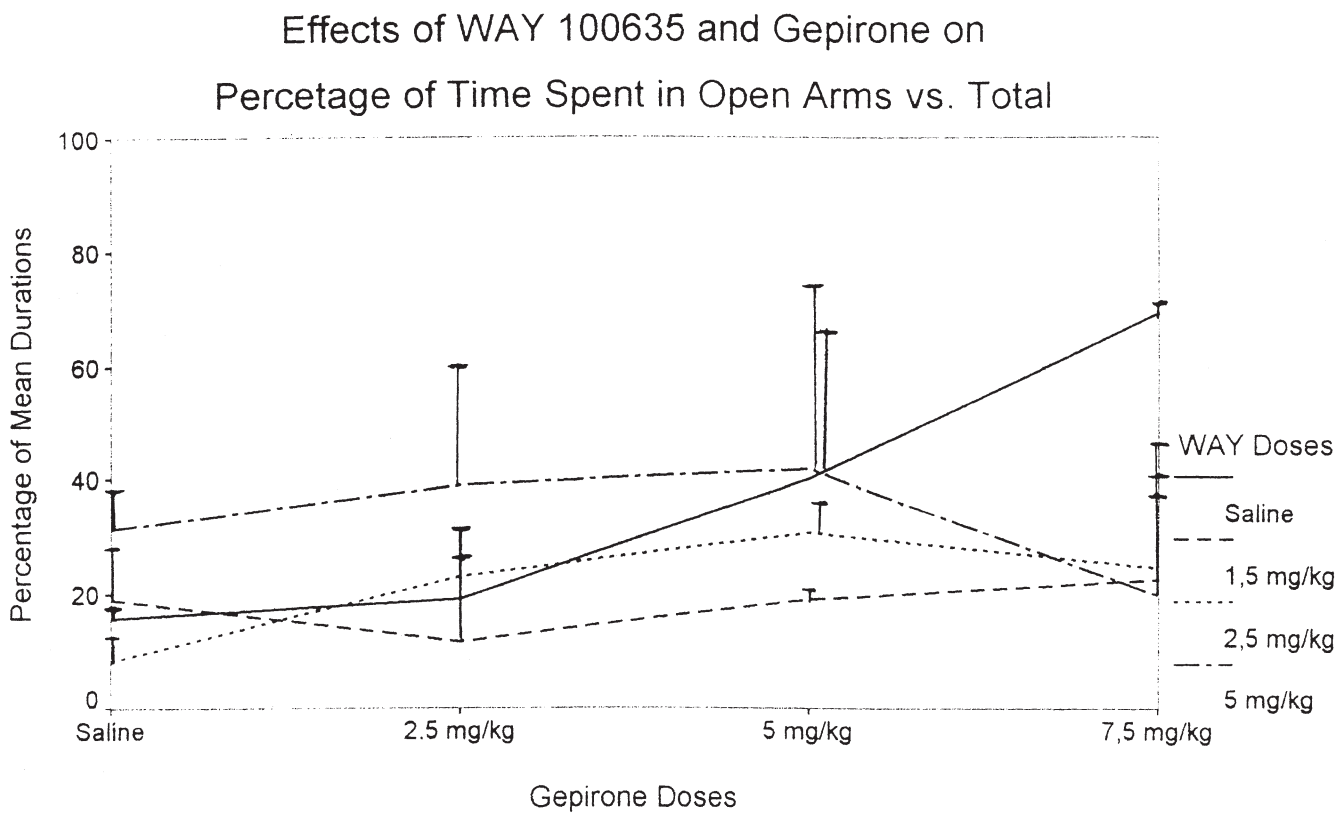

FIG. 6. Effects of (+)WAY 100635 and Gepirone on time spent in open arms. Time spent in open arms expressed as the percentage of total time (300 s) [time in open arms ÷ (time in open + time in closed arms + time in center) × 100]. Mean and SEM of each treatment group. Each line represents the effects of 0, 2.5, 5, and 7.5 mg/kg of Gepirone. The three different lines represent the additional effect of WAY pretreatment at the 0, 1.5, 2.5, and 5.0 mg/kg level.

shows that all doses of WAY prevented the rise produced by Gepirone [7.5 mg Gep vs. 7.5 mg Gep + various doses of WAY: 1.5 mg: $t(4) = 3.84$, $p < 0.05$; 2.5 mg: $t(4) = 2.97$, $p < 0.05$; 5 mg, $t(4) = 2.95$, $p < 0.05$].

Time spent on open and closed arms (Fig. 6). An increase in the percent of time spent in the open arms vs. total (open plus closed arms plus center) similarly reflects a decrease in anxiety. Gepirone increased the time spent in the open arms, the overall effect almost reaching significance, $F(3, 47) = 3.19$, $p = 0.08$, with follow-up analysis showing a highly significant effect of the largest dose of Gepirone [Sal vs. 7.5 mg Gep, $t(4) = 29.57$, $p < 0.001$]. Again, the largest dose of WAY also increased the time spent on the open arms [Sal vs. 5 mg WAY, $t(4) = 3.39$, $p < 0.05$]. Together with Gepirone, however, WAY at all dose levels counteracted the Gepirone induced increase [7.5 mg Gep vs. 7.5 mg Gep + various doses of WAY: 1.5 mg: $t(4) = 4.11$, $p < 0.05$; 2.5 mg; $t(4) = 3.47$, $p < 0.05$; 5 mg: $t(4) = 4.75$, $p < 0.01$].

Protected and unprotected head dips (Fig. 7). The percent of head dips performed from the protected areas of the open platform in relation to all head dips (protected and unprotected) is a measure of risk assessment. A decrease in protected dips reflects a decrease in anxiety. Although the great variability resulted in ANOVAs not being significant, follow-up analysis shows that the decrease shown on the graph with the largest dose of Gepirone is significant [Sal vs. 7.5 mg Gep, $t(4) = 3.75$, $p < 0.05$]. Furthermore, the largest dose of WAY, given alone also decreased protected head dips, suggesting anxiolytic action [Sal vs. 5 mg WAY, $t(4) = 2.68$, $p = 0.056$]. Combination of any dose of WAY with 7.5 mg Gepirone, however, completely inhibited the effect of Gepirone (7.5 mg Gep vs. 7.5 mg Gep + various doses of WAY: 2.5 mg: $t(4) = 2.96$, $p < 0.05$; 5 mg: $t(4) = 5.73$, $p < 0.01$]. An ANOVA performed on the direct measure of total protected head dips revealed a significant interaction between WAY and Gepirone, $F(9, 47) = 2.471$, $p < 0.05$.

Protected and unprotected stretches (Fig. 8). Similarly, stretches performed from the protected areas of the open arm are measures of risk assessment whose decrease reflects lowered anxiety. On this measure also (percent protected stretch vs. protected plus unprotected), ANOVAs failed to reach significance, but follow-up analysis showed the anxiolytic effect of 7.5 mg Gepirone was highly significant [Sal vs. 7.5 mg Gep, $t(4) = 8.47$, $p < 0.001$]. The decrease engendered by 5 mg WAY alone did not reach significance. Combination of any dose of WAY with 7.5 mg Gepirone completely inhibited the effect of Gepirone [7.5 mg Gep vs. 7.5 mg Gep + various doses of WAY: 1.5 mg: $t(4) = 2.84$, $p < 0.05$; 5 mg: $t(4) = 3.5$, $p < 0.05$]. ANOVA of the direct measure of protected stretches again supported this effect, because the interaction between WAY and Gepirone was significant, $F(9, 47) = 2.263$, $p < 0.05$.

Returns. The lessened necessity for risk assessment shown by an attenuation in protected behavior was supported by a decrease in the frequency of returning into a closed arm from

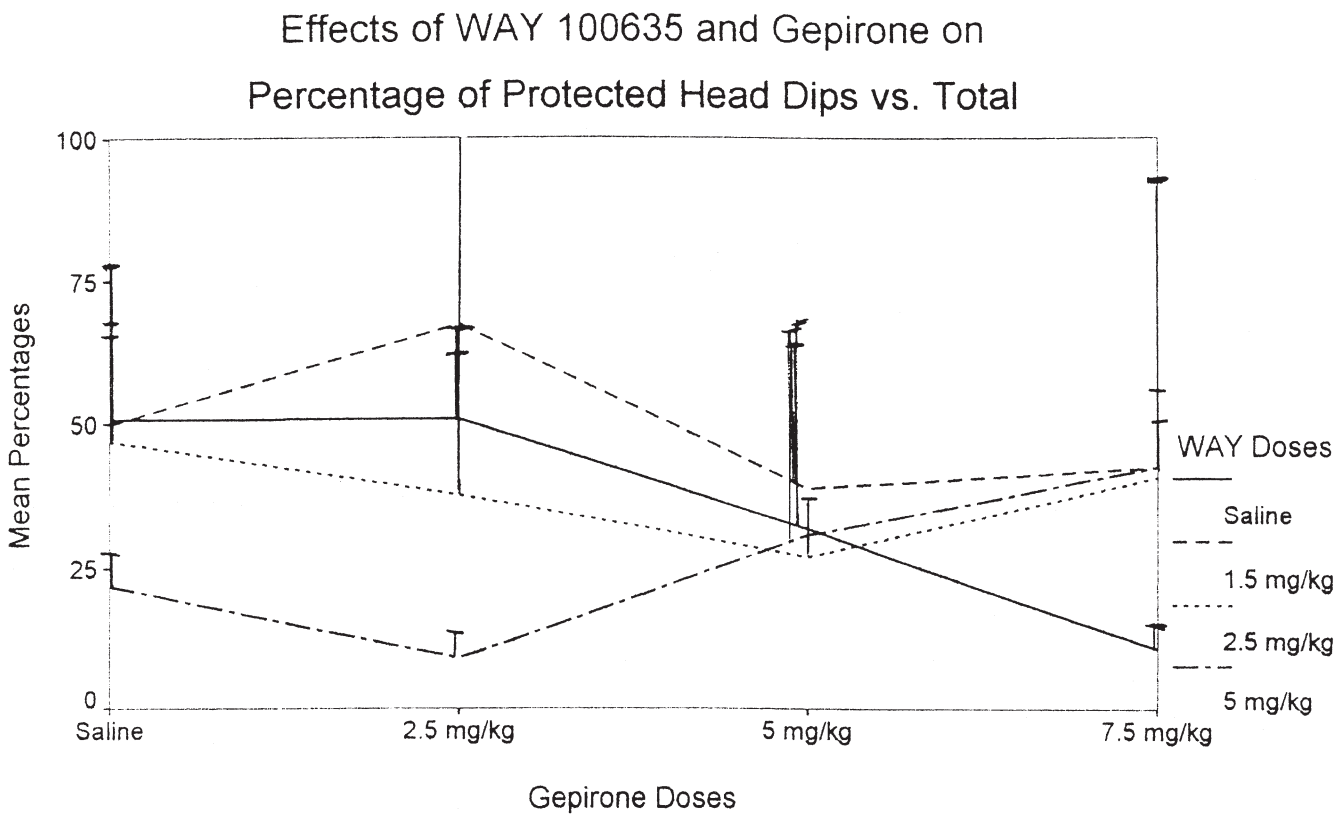

FIG. 7. Effects of (+)WAY 100635 and Gepirone on protected head dips. Protected head dips expressed as the percentage of total head dips (protected + unprotected). Mean and SEM of each treatment group. Each line represents the effects of 0, 2.5, 5, and 7.5 mg/kg of Gepirone. The three different lines represent the additional effect of WAY pretreatment at the 0, 1.5, 2.5, and 5.0 mg/kg level.

the center platform, occasioned by Gepirone at all dose levels, $F(3, 47) = 5.219$, $p < 0.01$. Although ANOVA showed no significant effect of WAY alone or in interaction with Gepirone, follow-up measures indicated that WAY inhibited Gepirone at the highest dose [7.5 mg Gep vs. 7.5 mg Gep + 1.5 mg WAY, $t(4) = 3.02$, $p < 0.05$].

DISCUSSION

It is now accepted that serotonin, via its action at 5-HT_{1A} as well as $_{1B}$ receptor sites, has an influence on intermale aggression (2,3,36,47). In the present experiment, the antiaggressive properties of the partial 5-HT_{1A} agonist, Gepirone, were confirmed in an experimental paradigm similar to the one reported previously (29). Latency to the first attack was increased in a dose-dependent manner, and there was a very clear dose-dependent inhibition of both overt attack and threat (tail rattling). As in the previous study, this was accompanied by increased exploratory sniffing and more time spent in a posture of watchfulness and attending, suggesting that the animal was less disturbed by the intrusion into his territory and, therefore, less inclined to impulsive attack. We suggested that this reduction in aggressiveness may have been concomitant with a decrease in anxiety, which seemed to fit with the well-known anxiolytic action of Gepirone and other (full or partial) 5-HT_{1A} agonists (32,39,41,44).

To ascertain whether Gepirone exerts anxiolytic effects in our strain of mice and under our laboratory conditions, all the animals were tested in an elevated plus-maze prior to being subjected to the resident–intruder test. This order of procedure was adopted so that differential experience in the aggression test would not confound the findings. Although the results were not very robust, there was a clear indication that Gepirone decreases anxiety, particularly at the higher dose levels. Both the classical measures (increased open-arm entries and time) and the more sensitive risk assessment measures described by Rodgers (41,42), i.e., head dips and attend stretch postures revealed an anxiolytic profile. It is interesting to note that the base levels of total, open- and closed-arm entries and time spent in open, closed arms, and center corresponded closely to those reported by Rodgers for his controls, even though he used a different strain of mice. Similarly, the total, protected, and unprotected head dips and stretches showed similar levels and ratios as Rodgers' controls. This supports the contention that our apparatus is indeed measuring the same parameters related to anxiety. The great individual variability between our subjects may be attributable to the stressful conditions before the elevated plus-maze test. Whereas Rodgers kept his animals in a dim light for 2 h prior to the test, our subjects were taken from the animal room into a brightly lit experimental room and given two injections and temperature recordings within the hour before the test. It is well known that results on the elevated plus-maze are sensitive to immediate pretest conditions (15,25,42). Finally, the weak anxiolytic response to low doses of a 5-HT_{1A} partial agonist administered systemically may be associated with a mixed response of pre- and postsynaptic receptors, as suggested by

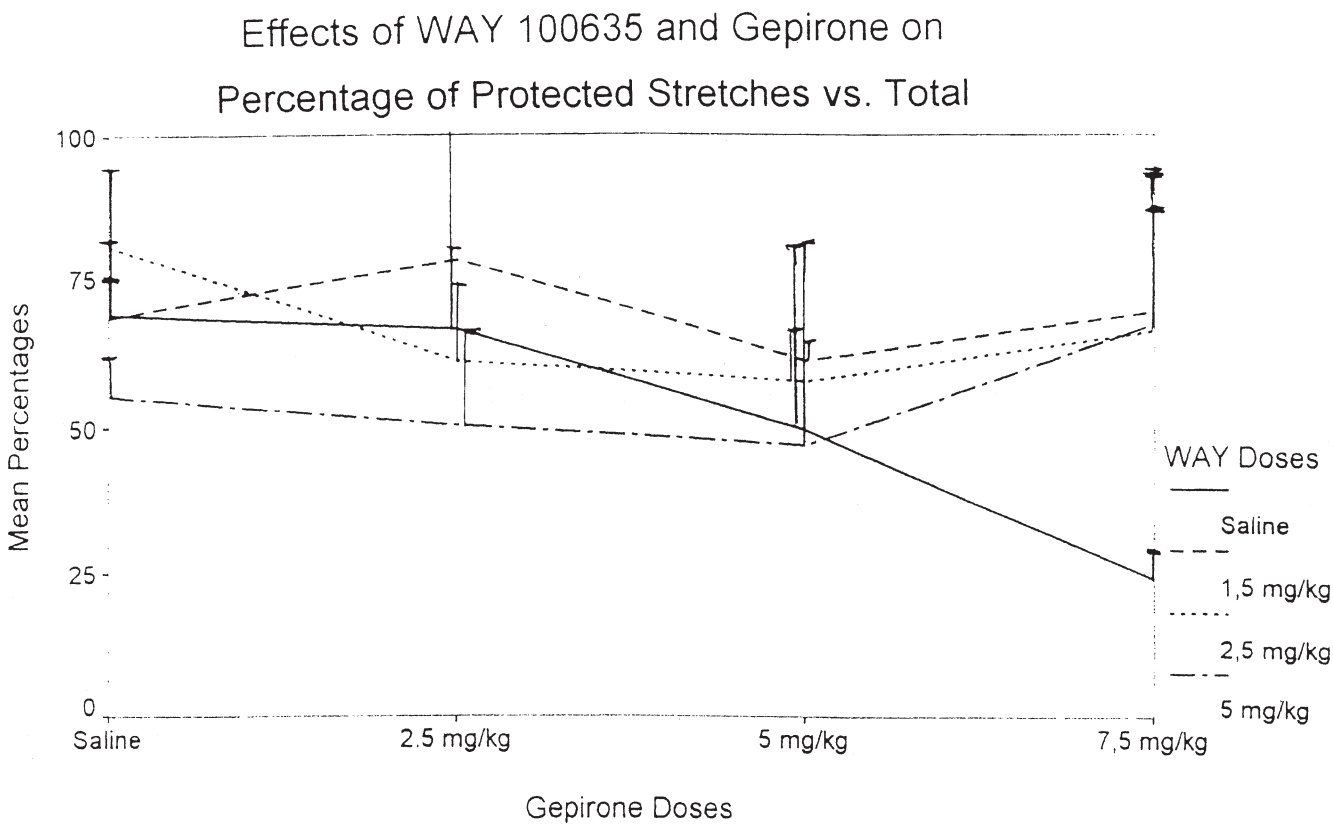

FIG. 8. Effects of (+)WAY 100635 and Gepirone on protected stretches. Protected stretches expressed as the percentage of total stretches (protected + unprotected). Mean and SEM of each treatment group. Each line represents the effects of 0, 2.5, 5, and 7.5 mg/kg of Gepirone. The three different lines represent the additional effect of WAY pretreatment at the 0, 1.5, 2.5, and 5.0 mg/kg level.

File (16) and Rodgers' group (6,7,22). Direct administration of the 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT, into the median raphe nucleus was anxiolytic, whereas injection into the dorsal hippocampus was anxiogenic (14,16). Systemic administration would be expected to produce mixed effects, with anxiolysis predominating.

A recent study with a new ligand of serotonin 5-HT$_{1A}$ receptors (S15535), which produces a net inhibition of serotonergic transmission by activation of presynaptic autoreceptors and blockade of postsynaptic receptors, showed that this compound had anxiolytic as well as antiaggressive properties (32). Moreover, these effects were counteracted by WAY 100635. These findings would seem to support our hypothesis regarding concomitant reduction of aggression and anxiety by a serotonin agonist.

The more recently developed compound, WAY 100635, was used in the present experiment instead of WAY 100135, which was used previously (29). Although the dose range overlapped, a lower dose was added because the newer compound is reported to be more potent (9,13,18,19,33,46). On the whole, the results were very similar with both compounds, although the newer substance had more consistent effects. This was to be expected, because WAY 100635 has been shown to be more selective as an 5-HT$_{1A}$ antagonist (1,11,17, 19,20). In the resident–intruder test of the present study, all doses of WAY counteracted the aggression inhibiting tendency of Gepirone on every measure without having any direct effect.

In the elevated plus-maze, WAY counteracted the anxiolytic effects of Gepirone, as was to be expected from an 5-HT$_{1A}$ antagonist. The largest dose of WAY (5 mg/kg), on the other hand, had a direct anxiolytic effect, a somewhat surprising findings but in line with a recent report (6).

Finally, with regard to body temperature, the well-documented hypothermic action of Gepirone was confirmed (8,30), as well as its reversal by 5-HT$_{1A}$ antagonists (19,26,45). There was a marked dose-dependent decrease in body temperature between the time of injection and temperature measurements (45 min), which was completely inhibited by all doses of WAY. A recent study on hypothermic action of 8-OH-DPAT showed that systemic administration of the drug resulted in maximum temperature reduction between 20 and 30 min after injection, which continued for up to 1 h, a response that was markedly attenuated by prior administration of WAY 100635 (34). In our previous study(29), where temperature was taken immediately before the first injection and after completion of the resident–intruder test, we showed that stress-induced hyperthermia was attenuated by Gepirone and restored by WAY. The present results indicate that this response could be attributed to the direct actions of both drugs on body temperature.

In conclusion, the present work support our contention that Gepirone exerts its antiaggressive effects through a reduction in anxiety by its agonistic action on 5-HT$_{1A}$ receptors, and that WAY 100635 counteracts these effects through its antagonist action on the same receptors.

REFERENCES

1. Assie, M. B.; Koek, W.: Effects of 5-HT_{1A} receptor antagonists on hippocampal 5-hydroxytryptamine levels: (S)-WAY 100135, but not WAY 100635, has partial agonists properties. Eur. J. Pharmacol. 304:15–21; 1996.
2. Bell, R.; Donaldson, C.; Gracey, D.: Differential effects of CGS 12066B and CP-94,253 on murine social and agonistic behaviour. Pharmacol. Biochem. Behav. 52:7–16; 1995.
3. Bell, R.; Mitchell, P. J.; Hobson, H.: Effects of the 5-HT1A antagonist (+)-WAY-100135 on murine social and agonistic behavior. Pharmacol. Biochem. Behav. 54:159–167; 1996.
4. Bosker, F. J.; deWinter, T. Y.; Klompmakers, A. A.; Westenberg, H. G.: Flesinoxan dose-dependently reduces extracellular 5-hydroxytryptamine (5-HT) in rat median raphe and dorsal hippocampus through activation of 5-HT_{1A} receptors. J. Neurochem. 66:2546–2555; 1996.
5. Bosker, F.; Vrinten, D.; Klompmakers, A.; Westenberg, H.: The effects of a 5-HT_{1A} receptor agonist and antagonist on the 5-hydroxytryptamine release in the central nucleus of the amygdala: A microdialysis study with flesinoxan and WAY 100635. Naunyn Schmiedebergs Arch. Pharmacol. 355:347–353; 1997.
6. Cao, B. J.; Rodgers, R. J.: Influence of 5-HT_{1A} receptor antagonism on plus-maze behaviour in mice, II. WAY 100635, SDZ 216-525 and NAN-190. Pharmacol. Biochem. Behav. 58:593–603; 1997.
7. Cao, B. J.; Rodgers, R. J.: Anxiolytic-like profile of p-MPPI, a novel 5-HT-1A receptor antagonist in the murine elevated plus-maze. Psychopharmacology (Berlin) 129:365–371; 1997.
8. Chojnacka, W. E.: Functional interaction between 5-HT_{1B} and 5-HT_{1A} or 5-HT_2 receptors in mice. Pol. J. Pharmacol. Pharm. 44:251–260; 1992.
9. Clarke, R. W.; Harris, J.; Houghton, A. K.: Spinal 5-HT-receptors and tonic modulation of transmission through a withdrawal reflex pathway in the decerebrated rabbit. Br. J. Pharmacol. 119:1167–1176; 1996.
10. Collinson, N.; Dawson, G. R.: On the elevated plus-maze the anxiolytic-like effects of the 5-HT(1A) agonist, 8-OH-DPAT, but not the anxiogenic-like effects of the 5-HT(1A) partial agonist, buspirone, are blocked by the 5-HT_{1A} antagonist, WAY 100635. Psychopharmacology (Berlin) 132:35–43; 1997.
11. Corradetti, R.; LePoul, E.; Laaris, N.; Hamon, M.; Lanfumey, L.: Electrophysiological effects of *N*-(-2-(4-(2-methoxypenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridiny1) ciclohexane carboxamine (WAY 100635) on dorsal raphe serotonergic neurons and CA1 hippocampal pyramidal cells in vitro. J. Pharmacol. Exp. Ther. 278:679–688; 1996.
12. Davids, E.; Lesch, K. P.: The 5-HT_{1A} receptor: A new effective principle in psychopharmacologic therapy? Fortschr. Neurol. Psychiatr. 64:460–472; 1996.
13. Davidson, C.; Stamford, J. A.: Evidence that 5-hydroxytryptamine release in rat dorsal raphe nucleus is controlled by 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} autoreceptors. Br. J. Pharmacol. 114:1107–1109; 1995.
14. DeAlmeida, R. M. M.; Giovenardi, M.; Charchat, H.; Lucion, A. B.: 8-OH-DPAT in different areas of brain have either anxiogenic or anxiolytic effects in female rats. Second Ethopharmacology Conference, Sopron; 1996 (published in Neurobiology 5:282; 1997).
15. Fernandes, C.; File, S. E.: The influence of open arm ledges and maze experience in the elevated plus maze. Pharmacol. Biochem. Behav. 54:31–40; 1996.
16. File, S. E.; Gonzalez, L. E.; Andrews, N.: Comparative study of pre- and postsynaptic 5-HT_{1A} receptor modulation of anxiety in two ethological animal tests. J. Neurosci. 16:4810–4815; 1996.
17. Fletcher, A.; Forster, E. A.; Bill, D. J.; Brown, G.; Cliffe, I. A.; Hartley, J. E.; Jones, D. E.; McLenachan, A.; Stanhope, K. J.; Critchley, D. J.; Childs, K. J.; Middlefell, V. C.; Lanfumey, L.; Coradetti, R.; Laporte, A. M.; Gozlan, H.; Hamon, M.; Dourish, C. T.: Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY 100635, a potent, selective and silent 5-HT_{1A} receptor antagonist. Behav. Brain Res. 73:337–353; 1996.
18. Fornal, C. A.; Metzler, C. W.; Gallegos, R. A.; Veasey, S. C.; McCreary, A. C.; Jacobs, B. L.: WAY 100635, a potent and selective 5-hydroxytryptamine$_{1A}$ antagonist, increases serotonergic neuronal activity in behaving cats: Comparison with (S)-WAY 100135. J. Pharmacol. Exp. Ther. 278:752–762; 1996.
19. Forster, E. A.; Cliffe, I. A.; Bill, D. J.; Dover, G. M.; Jones, D.; Reilly, Y.; Fletcher, A.: A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist, WAY 100635. Eur. J. Pharmacol. 281:81–88; 1995.
20. Gozlan, H.; Thibault, S.; Laporte, A. M.; Lima, L.; Hamon, M.: The selective 5-HT_{1A} antagonist radioligand [^{3}H]WAY 100635 labels both G-protein-coupled and free 5-HT_{1A} receptors in rat brain membranes. Eur. J. Pharmacol. 288:173–186; 1995.
21. Grant, E. C.; Mackintosh, J. H.: A description of the social postures of some common laboratory rodents. Behaviour 21:246–259; 1963.
22. Griebel, G.; Rodgers, R. J.; Perrault, G.; Sanger, D. J.: Risk assessment behaviour: Evaluation of utility in the study of 5-HT-related drugs in the rat elevated plus-maze test. Pharmacol. Biochem. Behav. 57:817–827; 1997.
23. Halasz, J.; Haller, J.; Makara, G. B.: The effect of social experience on the behavioral effectiveness of anxiolytic drugs. Second Ethopharmacology Conference, Sopron; 1996 (published in Neurobiology 5:292; 1997.)
24. Hjorth, S.; Bengtsson, H. J.; Milano, S.; Lundbert, J. F.; Sharp, T.: Studies on the role of 5-HT_{1A} autoreceptors and alfa 1-adrenoceptors in the inhibition of 5-HT release—I. BMY7378 and prazosin. Neuropharmacology 34:615–620; 1995.
25. Hogg, S.: A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol. Biochem. Behav. 54:21–30; 1996.
26. Koenig, J. I.; Meltzer, H. Y.; Gudelsky, G. A.: 5-Hydroxytryptamine$_{1A}$ receptor-mediated effects of buspirone, gepirone and ipsapirone. Pharmacol. Biochem. Behav. 29:711–715; 1988.
27. Krsiak, M.: Ethopharmacology: A historical perspective. Neurosci. Biobehav. Rev. 15:439–445; 1991.
28. Laporte, A. M.; Lima, L.; Gozlan, H.; Hamon, M.: Selective in vivo labelling of brain 5-HT_{1A} receptors by [^{3}H]WAY 100635 in the mouse. Eur. J. Pharmacol. 271:505–514; 1994.
29. Lopez Mendoza, D.; Aguilar Bravo, H.; Swanson, H. H.: Combined effects of Gepirone and (+) WAY 100135 on territorial aggression in mice. Pharmacol. Biochem. Behav. 61:1–8; 1998.
30. Martin, K. F.; Phillips, I.; Hearson, M.; Prow, M. R.; Heal, D. J.: Characterization of 8-OH-DPAT-induced hypothermia in mice as a 5-HT_{1A} autoreceptor response and its evaluation as a model to selectively identify antidepressants. Br. J. Pharmacol. 107:15–21; 1992.
31. Mendlin, A.; Martin, F. J.; Rueter, L. E.; Jacobs, B. L.: Neuronal release of serotonin in the cerebellum of behaving rats: An in vivo microdialysis study. J. Neurochem. 67:617–622; 1996.
32. Millan, M. J.; Hjorth, S.; Samanin, R.; Schreiber, R.; Jaffard, R.; DeLadonchamps, B.; Veiga, S.; Goument, B.; Peglion, J. L.; Spedding, M.; Brocco, M.: S 15535, a novel benzodioxopiperazine ligand of serotonin (5-HT)$_{1A}$ receptors: II. Modulation of hippocampal serotonin release in relation to potential anxiolytic properties. J. Pharmacol. Exp. Ther. 282:148–161; 1997.
33. Mundey, M. K.; Fletcher, A.; Marsden, C. A.: Effects of 8-OHDPAT and 5-HT_{1A} antagonists WAY100135 and WAY100635, on guinea-pig behaviour and dorsal raphe 5-HT neurone firing. Br. J. Pharmacol. 117:750–756; 1996.
34. Patel, S.; Hutson, P. H.: Effects of galanin on 8-OH-DPAT induced decrease in body temperature and brain 5-HT metabolism in the mouse: Eur. J. Pharmacol. 317:197–204; 1996.
35. Pecknold, J. C.; Luthe, L.; Scott-Fleury, M. H.; Jenkins, S.: Gepirone and the treatment of panic disorder: An open study. J. Clin. Psychopharmacol. 13:145–149; 1993.
36. Ramboz, S.; Saudou, F.; Amara, D. A.; Belzung, C.; Segu, L.; Misslin, R.; Buhot, M. C.; Hen, R.: 5-HT_{1B} receptor knock out—Behavioral consequences. Behav. Brain Res. 73:305–312; 1996.
37. Remy, S. M.; Schreiber, R.; Dalmus, M.; DeVry, J.: Somatodendritic 5-HT_{1A} receptors are critically involved in the anxiolytic effects of 8-OH-DPAT. Psychopharmacology (Berlin) 125:89–91; 1996.

38. Rickels, K.; Schweizer, E.; DeMartinis, N.; Mandos, L.; Mercer, C.: Gepirone and diazepam in generalized anxiety disorder: A placebo controlled trial. J. Clin. Psychopharmacol. 17:272–277; 1997.
39. Rodgers, R. J.: Animal models of "anxiety": Where next? Behav. Pharmacol. 8:477–496; 1997.
40. Rodgers, R. J.: Effects of benzodiazepine and 5-HT receptor ligands on aggression and defence in animals. In: Rodgers, R. J.; Cooper, S. J., eds. 5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines: Their comparative behavioural pharmacology. London: John Wiley & Sons; 1991:195–231.
41. Rodgers, R. J.; Cole, J. C.; Cobain, P.; Doran, P. J.; Eells, J. R.; Wallis, P.: Anxiogenic-like effects of fluprazine and eltoprazine in the mouse-elevated plus-maze: Profile comparisons with 8-OH-DPAT, CGS 12066B, TFMPP and mCPP. Behav. Pharmacol. 3: 621–634; 1992.
42. Rodgers, R. J.; Dalvi, A.: Anxiety, defence and the elevated plus-maze. Neurosci. Biobehav. Rev. 21:801–810; 1997.
43. Sanchez, C.; Arnt, J.; Moltzen, E. K.: The antiaggressive potency of (−)-penbutolol involves both 5-HT_{1A} and 5-HT_{1B} receptors and beta-adrenoceptors. Eur. J. Pharmacol. 297:1–8; 1996.
44. Sasa, M.: A new approach to innovating selective anxiolytics: Pharmacological profile of a novel 5-HT_{1A} agonist (tandospirone). Nihon Shinkei Seishin Yakurigaku Zasshi. 17:53–59; 1997.
45. Scott, P. A.; Chou, J. M.; Tang, H.; Frazer, A.: Differential induction of 5-HT_{1A}-mediated responses in vivo by three chemically dissimilar 5-HT_{1A} agonists. J. Pharmacol. Exp. Ther. 270:198–208; 1994.
46. Sharp, T.; Umbers, V.; Hjorth, S.: The role of 5-HT_{1A} autoreceptors and alpha 1-adrenoceptors in the inhibition of 5-HT release—II. NAN-190 and SDZ 216-525. Neuropharmacology 35:735–741; 1996.
47. Simon, N. G.: Testosterone-serotonin interactions in aggression. Second Ethopharmacology Conference. Sopron, 1996 (published in Neurobiology 5:322; 1997).
48. Stanhope, K. J.; Dourish, C. T.: Effects of 5-HT_{1A} receptor agonists, partial agonists and a silent antagonist on the performance of the conditioned emotional response test in the rat. Psychopharmacology (Berlin) 128:293–303; 1996.
49. Stefanski, R.; Palejko, W.; Kostowski, W.; Plaznik, A.: The comparison of benzodiazepine derivatives and serotonergic agonists and antagonists in two animal models of anxiety. Neuropharmacology 31:1251–1258; 1992.
50. Tornatzky, W.; Miczek, K. A.: Alcohol, anxiolytics and social stress in rats. Psychopharmacology (Berlin) 121:135–144; 1995.
51. Vale, A. L.; Montgomery, A. M.: Social interaction: Responses to chlordiazepoxide and the loss of isolation-reared effects with paired-housing. Psychopharmacology (Berlin) 133:127–132; 1997.
52. Valzelli, L.: Aggression and violence: A biological essay of the distinction. In: Valzelli, L.; Morgese, I., eds. Aggression and violence: A psychobiological approach. Milan: Ediz. St. Vincent; 1981:39–60.
53. Vivian, J. A.; Miczek, K. A.: Diazepam and Gepirone selectively attenuate either 20–32 or 32–64 kHz ultrasonic vocalizations during aggressive encounters. Psychopharmacology (Berlin) 112:66–73; 1993.
54. Xu, L.; Anwyl, R.; de Vry, J.; Rowan, M. J.: Effect of repeated ipsapirone treatment on hippocampal excitatory synaptic transmission in the freely behaving rat: Role of 5-HT_{1A} receptors and relationship to anxiolytic effect. Eur. J. Pharmacol. 323:59–68; 1997.