ELSEVIER

Pharmacology Biochemistry & Behavior, Vol. 55, No. 2, pp. 303–308, 1996

0091-3057/96 $15.00 + .00

PII S0091-3057(96)00088-3

The 5-HT_{1A} Receptor Is Not Involved in Emotional Stress-Induced Rises in Stress Hormones

LUCIANNE GROENINK,*[1] JAN MOS,*
JAN VAN DER GUGTEN* AND BEREND OLIVIER*†

*Department Psychopharmacology, Faculty of Pharmacy and Rudolf Magnus Institute for Neurosciences, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands
†CNS-Research, Solvay Duphar B.V., Weesp, The Netherlands

Received 31 October 1995; Revised 3 March 1996; Accepted 3 March 1996

GROENINK, L., J. MOS, J. VAN DER GUGTEN AND B. OLIVIER. *The 5-HT_{1A} receptor is not involved in emotional stress-induced rises in stress hormones.* PHARMACOL BIOCHEM BEHAV **55**(2) 303–308, 1996.—To determine whether emotional stress-induced rises in stress hormone levels are mediated by activation of 5-HT_{1A} receptors, we studied the effects of the selective 5-HT_{1A} receptor antagonist, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride (WAY-100635) on plasma ACTH, corticosterone, prolactin, and glucose levels in the conditioned ultrasonic vocalisation (USV) model in adult rats. The effects of WAY-100635 on USVs were also investigated in this paradigm. WAY-100635 (0.3, 1, and 3 mg/kg SC) had no clear effects on basal plasma ACTH, corticosterone, and glucose levels, but the 3 mg/kg dose significantly increased the plasma prolactin levels. The increases in plasma ACTH, corticosterone, and prolactin levels induced by the USV procedure were not affected by WAY-100635. This indicates that the 5-HT_{1A} receptor does not play a major role in the distress-induced activation of the hypothalamic–pituitary–adrenal axis and prolactin secretion. The USVs were significantly enhanced by low doses of WAY-100635 (0.03 and 0.3 mg/kg SC), whereas higher doses (1.0 and 3.0 mg/kg SC) had no effect. These findings suggest that blockade of 5-HT_{1A} receptors during stress may enhance the behavioural stress-response. **Copyright © 1996 Elsevier Science Inc.**

5-HT_{1A} receptor antagonist Emotional stress ACTH Corticosterone Prolactin Glucose WAY-100635

A variety of stressors has been shown to enhance the activity of the hypothalamic–pituitary–adrenal (HPA) axis (1). 5-HT receptor agonists also increase the activity of the HPA axis. 5-HT_{1A} receptor agonists (11,13), as well as 5-HT_2 [see (5) for review] and probably 5-HT_3 receptor agonists (4,21), induce rises in plasma ACTH and corticosterone levels. Not only the HPA axis but also prolactin secretion and plasma catecholamine levels are influenced both by stress and by 5-HT_{1A} receptor agonists (7,24). As the neuronal 5-HT system is activated during stress (5), it could be hypothesized that 5-HT_{1A} receptors are involved in the stress-induced activation of the HPA axis.

If the stress-induced activation of the HPA axis and prolactin secretion is mediated by activation of 5-HT_{1A} receptors, a selective 5-HT_{1A} receptor antagonist may block stress-induced rises in plasma ACTH, corticosterone, and/or prolactin levels. To test this hypothesis, we examined the effects of the selective 5-HT_{1A} receptor antagonist, WAY-100635 (10), in the conditioned ultrasonic vocalisation (USV) model in adult rats (17). In this conditioned USV test, rats produce USVs in association with a prior aversive event. USVs are elicited by reintroducing adult male rats into the environment where they previously received inescapable shocks. We used this paradigm because we were interested in conditioned emotional stress rather than in physical stress. Also, the pharmacological characteristics of this paradigm are well studied (17). As such, we have some clues about processes underlying the stress effect. Moreover, considering the outcome of pharmacological studies, USV paradigms may have predictive validity for anxiolytic drug effects (2,8,14,17).

METHOD

Animals

Male Wistar rats (Harlan, CPB, Zeist, The Netherlands), weighing 200–250 g, were used. They were housed individually

[1] To whom requests for reprints should be addressed.

under controlled environmental conditions (12 L:12 D cycle, lights on at 0700 h, temperature 21 ± 2°C, and humidity 60%). Standard rat chow and water were freely available.

Apparatus and Procedure

A circular nontransparent cage (20 cm diameter, 11.5 cm high) without bottom was placed on a grid floor connected to a shock generator and scrambler. The ultrasone sensitive microphone (Bruel and Kjaer 4135) was positioned in the ceiling of the cage and the whole cage was placed in a sound-attenuating chamber. The ultrasounds were recorded as described before (17).

On day 1, the rats received six randomly distributed inescapable shocks (0.8 mA, 8 s each) during a 7-min period. The intershock interval varied between 30 and 90 s. On day 2, one reminder shock (0.8 mA, 8 s) was given (2 min in the test cage). Thirty minutes later the rats were returned to the test cage and their USV production was measured in a 10-min test session (pretest), while no current was administered. Rats (n = 32) were matched based on the USV production in the pretest and divided over eight groups (n = 4 per group), such that rats of one group were comparable with regard to their USV production. Each group received a USV category number (1 = high number of USV in pretest, 8 = low number of USV in pretest), and each of the four treatments were tested within one group, allowing statistical analyses within and between groups. On day 3, no shocks were given at all, in contrast to the standard procedure. This was done to exclude possible interference of direct shock effects with emotional effects. The rats were placed in the test cage for 2 min. Subsequently, they were injected (0, 0.3, 1.0, or 3.0 mg/kg SC). Thirty minutes later the rats were returned to the test cage and USVs were recorded for 10 min. Immediately after this test session the rats were decapitated. To measure the effects of WAY-100635 (0, 0.3, 1, and 3 mg/kg SC) under basal nonstress conditions, a home cage control rat was injected parallel to a USV-trained rat. Both rats received the same drug treatment. All animals were decapitated 40 min after the injection in a separate room. Blood was collected in ice-cooled tubes containing 0.21 M EDTA (50 μl/ml blood). Plasma was separated by centrifugation (3000 rpm for 10 min at 4°C) and stored at −80°C until assayed.

As the basal levels of USVs were extremely low, we also tested the effects of WAY-100635 (0, 0.03, 0.3, and 3.0 mg/kg SC) in the standard USV test procedure (17). That is, prior to the drug injection on day 3, the rats received one reminder shock (0.8 mA, 8 s). This standard test procedure induced normal USV levels in the control group (17). In this experiment stress hormones were not measured. All experiments were performed between 0830 and 1230 h. The experiments were approved by the ethical committee of Solvay Duphar.

Chemical Determinations

Plasma ACTH and prolactin were determined in duplicate using a double antibody radioimmuno assay (RIA) for rat ACTH (Diagnostic Products Corporation BV, Apeldoorn, The Netherlands; inter- and intraassay variablities were 9.1 and 5.9%, sensitivity 6.7 pg/ml), and rat prolactin (Amersham, UK; inter- and intraassay variablities were 9.7 and 3.2%, sensitivity 1.4 ng/ml). Plasma corticosterone levels were measured in duplicate with a coat-a-count RIA (Diagonistic Products Corporation BV, Apeldoorn, The Netherlands; inter- and intraassay variablities were 8.3 and 6.8%, sensitivity 0.6 μg/dl). Plasma glucose levels were determined using a hexokinase UV test (Hoffmann–LaRoche, Diagnostica, Mijdrecht, The Netherlands).

Drugs

WAY-100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride) was synthesized by Solvay-Duphar B.V., The Netherlands). WAY-100635 was dissolved in destilled water and given subcutaneously (SC) in a volume of 2 ml/kg.

Statistics

Statistical analysis of the neuroendocrine data was performed by a two-way analysis of variance (ANOVA) with WAY-100635 treatment and USV stress as between factors. Treatment effects were further analysed with Duncan's new multiple range test. Comparisons between nonstressed and stressed rats were made using Student's t-test.

The behavioral data were analyzed according to Molewijk et al. (17). An ANOVA was used with WAY-100635 and USV category number as explanatory factor. USV category number is used as within factor to reduce the effect of interanimal variations. Further comparisons were made using Student's t-test. For all tests the criterion of significance was set at $p < 0.05$.

RESULTS

Exposure of the rats to the USV procedure resulted in significant increases in plasma ACTH, corticosterone, and prolactin levels, compared to nonstressed rats. The plasma glucose levels were not altered by the USV procedure (Fig. 1).

WAY-100635 treatment had no significant effect on plasma ACTH concentrations, and the ACTH response to stress was not altered by WAY-100635.

Under basal nonstress conditions the 1.0 mg/kg dose of WAY-100635 increased the plasma corticosterone levels as compared to vehicle treatment. In stressed rats, the highest dose of WAY-100635 (3.0 mg/kg SC) increased the plasma corticosterone levels as compared to those of vehicle-treated stressed rats.

The plasma prolactin levels were significantly increased with the highest dose of WAY-100635, both under nonstress and stress conditions. WAY-100635 did not alter the prolactin response to USV stress.

WAY-100635 treatment had no significant effect on plasma glucose concentrations, nor did it alter the effects on plasma glucose levels following the USV stress.

The lowest dose WAY-100635 (0.3 mg/kg SC) significantly augmented the number of ultrasonic vocalisations as compared to that of control rats (Fig. 2A). Higher doses of WAY-100635 did not significantly enhance the number of ultrasonic calls. These effects of WAY-100635 were reflected in the duration of calls (Fig. 2B).

In the second USV experiment, using the standard USV procedure, WAY-100635 (0.03, 0.3, and 3.0 mg/kg SC) had no significant effect on the number of USVs (Fig. 3A). However, the duration of calls was significantly increased by 0.03 and 0.3 mg/kg WAY-100635 (Fig. 3B).

DISCUSSION

Under basal nonstress conditions, WAY-100635 does not clearly affect the HPA axis activity. The plasma ACTH levels were not altered by WAY-100635, and although there is a

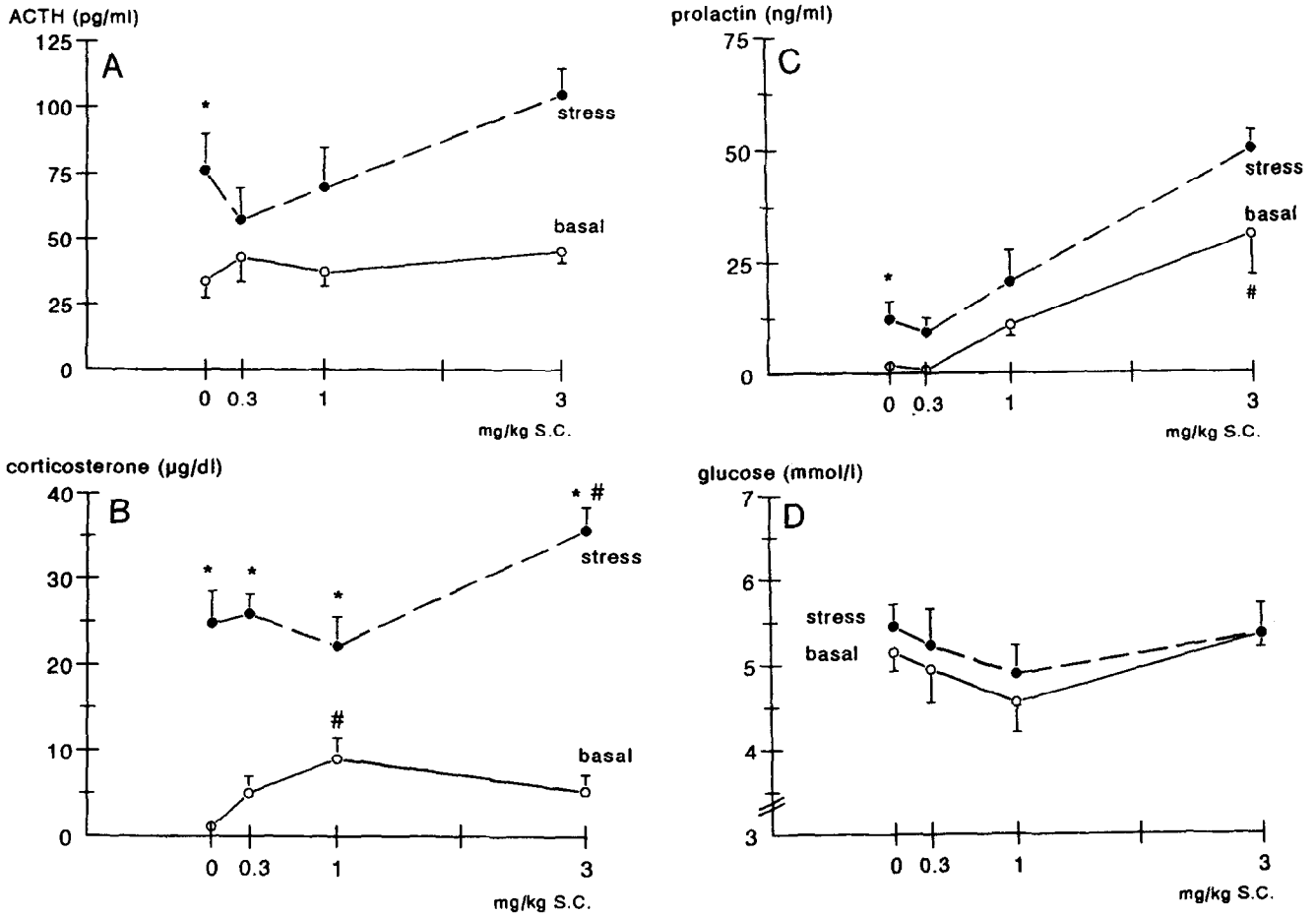

FIG. 1. Effects of WAY-100635 on plasma ACTH (A), corticosterone (B), prolactin C), and glucose (D) levels under basal conditions (open circles) and in the USV procedure (filled circles). Data are given as mean ± SEM. Each treatment group consisted of seven to eight rats. A main stress effect (indicated as * in vehicle condition) was found for plasma ACTH, $F(1, 56) = 26.4$, $p < 0.000$), corticosterone, $F(1, 56) = 152$, $p < 0.0001$, and prolactin levels, $F(1, 56) = 12.2$, $p = 0.001$. ANOVA yielded significant main treatment effects for plasma corticosterone, $F(3, 56) = 3.1$, $p = 0.034$, and prolactin levels, $F(3, 56) = 23.0$, $p < 0.0001$. The treatment × stress interaction was only significant for plasma corticosterone, $F(3, 56) = 4.0$, $p = 0.012$. *$p < 0.05$ compared to basal levels (Student's t-test), #$p < 0.05$ compared to corresponding vehicle (Duncan's multiple range test).

small but significant increase in plasma corticosterone levels after injection with 1.0 mg/kg WAY-100635, this increase is not reflected in the plasma ACTH levels. The discrepancy between the effects of WAY-100635 on ACTH and corticosterone levels might be due to a time effect. The effect on plasma ACTH may already have subsided 1 h after WAY-100635 administration. However, Critchley and co-workers (6) measured plasma ACTH levels every 10 min after intravenous WAY-100635 administration and also did not find an effect of WAY-100635 on plasma ACTH levels.

Plasma prolactin levels were strongly enhanced by 3.0 mg/kg SC WAY-100635. This may seem surprising, as 5-HT$_{1A}$ receptor agonists also enhance prolactin secretion (11,15). Moreover, it has recently been shown that (−)-pindolol, a 5-HT$_{1A, 1B}$, and β-adrenergic receptor antagonist, reduced plasma prolactin levels in humans (20). However, it should be considered that the 3 mg/kg dose of WAY-100635 is rather high regarding the doses at which WAY-100635 exerts its 5-HT$_{1A}$ receptor blocking activity in antagonist studies (9,10). Therefore, it is doubtful whether the effect of WAY-100635 on plasma prolactin levels is due to activition of 5-HT$_{1A}$ receptors or to another mechanism in WAY-100635. Although the selectivity of WAY-100635 for 5-HT$_{1A}$ receptors is at least more than 75- to a 100-fold higher than for other receptors (5-HT$_7$, α_1 adrenoceptor, 5-HT$_{1B}$, 5-HT$_{2C}$, and dopamine D_1, D_2, D_4) (10), the 3.0 mg/kg dose of WAY-100635 may have affected some of these receptors as well.

WAY-100635 had no significant effect on plasma glucose levels. As is shown in Fig. 1D, the variance between animals is rather large, which complicates reliable interpretation of the data.

Exposure of the rats to the USV test resulted in significant rises in plasma ACTH, corticosterone, and prolactin levels. WAY-100635 treatment did not alter the stress-induced elevations in these parameters. Apparently, blockade of 5-HT$_{1A}$ receptors does not result in blunted stress hormone reponses. Haleem and co-workers (13) reported that the rise in plasma corticosterone induced by tube restraint was completely prevented by the 5-HT$_{1A}$, 5-HT$_{1B}$, and β-adrenergic receptor antagonist, (−)-pindolol. This discrepancy between the two studies may indicate that the involvement of 5-HT$_{1A}$ receptors in stress-induced hormone secretion depends on the kind of stressor, for instance, physical (restraint) or emotional (USV

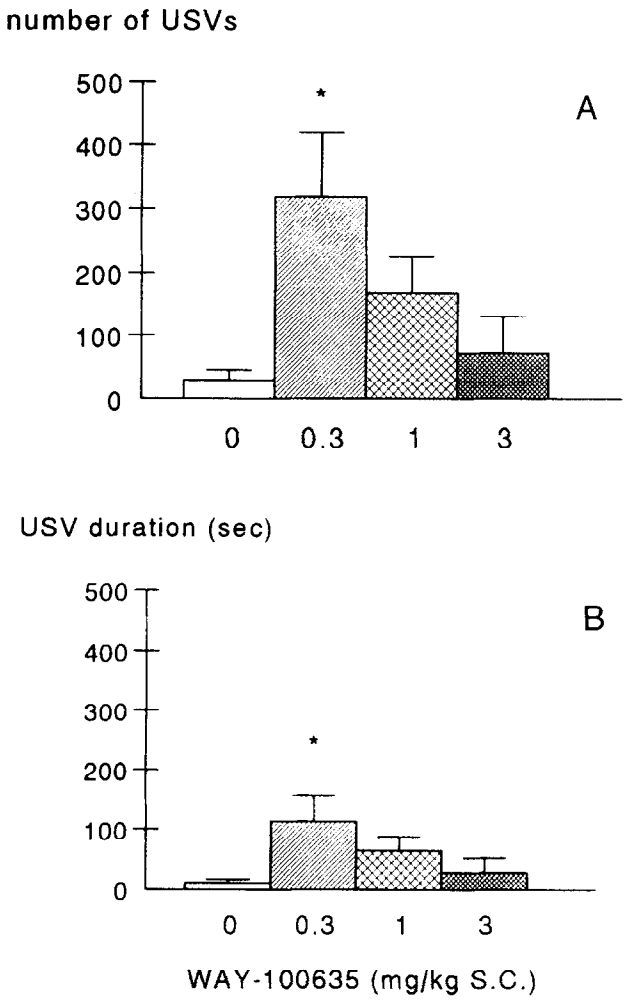

FIG. 2. Effects of WAY-100635 on the number (A) and the duration (B) of USVs. Each treatment group consisted of seven to eight rats. Data are given as mean (± SEM). ANOVA yielded significant main effects of treatment for the number, $F(3, 21) = 9.15$, $p < 0.001$, and duration, $F(3, 21) = 6.9$, $p = 0.002$, of USVs. *$p < 0.05$ compared to vehicle-treated rats.

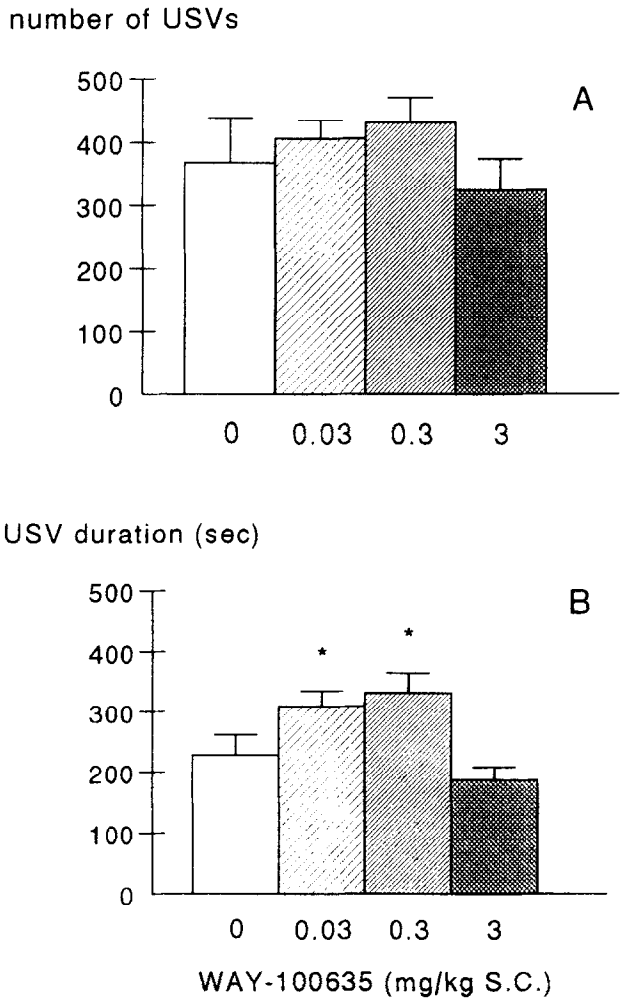

FIG. 3. Effects of WAY-100635 on the number (A) and the duration (B) of USVs in the standard USV procedure. Each treatment group consisted of eight rats. Data are given as mean (± SEM). ANOVA yielded a significant main effect of treatment for the duration of USVs, $F(3, 21) = 6.7$, $p = 0.002$). *$p < 0.05$ compared to vehicle-treated rats.

procedure). The fact that the physical stress is applied directly, whereas the conditioning procedure itself may have altered 5-HT receptor sensitivity (16) and/or the sensitivity of the HPA axis (25), may contribute to differences between these types of stressors. Finally, it may be possible that the suppression of stress-induced corticosterone secretion by (−)-pindolol was due to β-adrenergic receptor blockade, instead of 5-HT_{1A} receptor blockade.

The highest dose of WAY-100635 induced a significant rise in plasma corticosterone concentrations in stressed rats as compared to vehicle-treated stressed rats. This effect was reflected in the plasma ACTH levels, although here the effect was only significant compared to the corresponding home cage control group. Apparently, combining high doses of WAY-100635 with stress may result in exaggerated activation of the HPA-axis.

The effects of WAY-100635 on plasma prolactin levels in stressed rats show a parallel shift compared to the effects in non stressed rats. This clearly demonstrates that the 5-HT_{1A} receptor is not involved in mediating stress-induced rises in prolactin secretion.

WAY-100635 enhanced the number of USVs in this study. As anxiolytic drugs like benzodiazepines and 5-HT_{1A} receptor agonists consistently reduce USVs in several USV paradigms, measurement of distress vocalizations has been proposed as screening method for anxiolytic drug effects (2,8,14,17). As a reduction in USVs in these paradigms is interpreted as anxiolytic effect, it follows that an enhancement of USVs could be interpreted as anxiogenic-like effect. Although the results of the first experiment were not fully replicated in the standard USV procedure, 0.3 mg/kg WAY-100635 enhanced the duration of calls in both experiments. Moreover, in the second experiment the 0.03 mg/kg dose, which was not tested in the first experiment, also enhanced the duration of USVs. This may indicate that blockade of 5-HT_{1A} receptors during stress enhances the behavioral stress response. Probably the effect on the number of USVs was more pronounced in the first experiment because of the relatively low USV level induced

by the adapted USV procedure. The basal level of vocalization is extremely low compared to previous studies (17). This may be due to the fact that in the adapted procedure no reminder shock was given on the test day. As far as we know, no anxiogenic-like effects have been reported for WAY-100635 before. WAY-100635 had anxiolytic effects in the light-dark box and the elevated plus-maze model in mice (3). In ferrets, WAY-100635 also produced anxiolytic-like effects (22). In rats, however, no effects were found in the conditioned emotional response test (23). On the other hand, another 5-HT_{1A} receptor antagonist, WAY-100135 (*N-tert*-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenyl propanamide dihydrochloride), also exerted anxiogenic-like effects again displaying a bell-shaped dose–response curve, in the rat pup vocalization test (19). It would be interesting to test WAY-100635 in other animal models with a relatively low anxiety level to see whether the enhancement of USVs may reflect anxiogenic-like effects. Alternatively, it could be possible that 5-HT_{1A} receptor antagonists have a nonspecific action on USVs, as the anxiogenic-like effects of WAY-100635 and WAY-100135 sofar were only found in USV paradigms. However, it should be noted that USV paradigms seem relatively insensitive to confounding drug effects, like motor relaxant, sedative, and amnesic effects, as recently discussed by De Vry and co-workers (8).

The increase in USVs found with 0.03 and 0.3 mg/kg WAY-100635 was not observed with higher doses. Electrophysiological and microdialysis studies indicate that WAY-100635 enhances the activity of the serotonergic system during periods of high 5-HT neuronal activity (12,18). As stress is supposed to be associated with increased activity of the serotonergic system (5), this may suggest that lower doses of WAY-100635 further enhance 5-HT neurotransmission during stress by blocking presynaptic 5-HT_{1A} receptors, which subsequently may result in an increase in USVs. Apparently, higher doses of WAY-100635 either activate other mechanisms or change the balance between pre- and postsynaptic blockade of 5-HT_{1A} receptors (9).

In conclusion, our study with the selective and silent 5-HT_{1A} receptor antagonist WAY-100635 indicates that the 5-HT_{1A} receptor does not play a major role in the stress-induced HPA axis activation nor in stress-induced prolactin secretion. However, the 5-HT_{1A} receptor does seem to be involved in the regulation of behavioral processes, as blockade of the 5-HT_{1A} receptor during stress enhances the behavioral stress response.

ACKNOWLEDGEMENTS

We thank Ruud van Oorschot, Ellen Molewijk, Theo Hijzen and Wim van der Wal for their excellent contributions.

REFERENCES

1. Axelrod, J.; Reisine, T. D. Stress hormones: Their interaction and regulation. Science 224:452–459; 1983.
2. Barrett, J. E. Animal behaviour models in the analysis and understanding of anxiolytic drugs acting at serotonin receptors. In: Olivier, B.; Mos, J.; Slangen, J. L., eds. Animal models in psychopharmacology. Basel: Birkhäuser Verlag; 1991:37–52.
3. Bill, D. J.; Fletcher, A. A comparison of the potencies of the novel silent 5-HT_{1A} antagonist, WAY-100635, in functional and anxiety models. Behav. Pharmacol. 5S:77; 1994.
4. Calogero, A. E.; Bagdy, G.; Burrello, N.; Polosa, P.; D'Agata, R. Role of serotonin$_3$ receptors in the control of adrenocorticotropic hormone release from rat pituitary cell cultures. Eur. J. Endocrinol. 133:251–254; 1995.
5. Chaouloff, F. Physiopharmacological interactions between stress hormones and central serotonergic systems. Brain Res. Rev. 18:1–32; 1993.
6. Critchley, D. J. P.; Childs, K. J. Dourish, C. T. Inhibition of the 8-OH-DPAT-induced elevation of plasma corticotrophin by the 5-HT_{1A} receptor antagonist WAY-100635. Eur. J. Pharmacol. 264:95–97; 1994.
7. De Boer, S. F.; Slangen, J. L.; Van der Gugten, J. Effects of buspirone and chlordiazepoxide on plasma catecholamine and corticosterone levels in stressed and non stressed rats. Pharmacol. Biochem. Behav. 38:299–308; 1991.
8. De Vry, J.; Benz, U.; Schreiber, R.; Traber, J. Shock-induced ultrasonic vocalization in young adult rats: A model for testing putative anti-anxiety drugs. Eur. J. Pharmacol. 249:331–339; 1993.
9. Fletcher, A.; Bill, D. J.; Cliffe, I. A.; Forster, E. A.; Jones, D.; Reilly, Y. A pharmacological profile of WAY-100635, a potent and selective 5-HT_{1A} receptor antagonist. Br. J. Pharmacol. 112:91P; 1994.
10. Forster, E. A.; Cliffe, I. A.; Bill, D. J.; Dover, G. M.; Jones, D.; Reilly, Y.; Fletcher, A. A pharmacological profile of the silent 5-HT_{1A} receptor antagonist, WAY-100635. Eur. J. Pharmacol. 281:81–88; 1995.
11. Groenink, L.; Van der Gugten, J.; Verdouw, P. M.; Maes, R. A. A.; Olivier, B. The anxiolytic effects of flesinoxan, a 5-HT_{1A} receptor agonist, are not related to its neuroendocrine effects. Eur. J. Pharmacol. 280:185–193; 1995.
12. Gurling, J.; Shilliam, C.; Routledge, C. R.; Dourish, C. T. Increased 5-HT release induced by a 5-HT_{1A} antagonist is determined by arousal state (abstract book). J. Psychopharmacol. A13; 1994.
13. Haleem, D. J.; Kennett, G. A.; Whitton, P. S.; Curzon, G. 8-OH-DPAT increases corticosterone but not other 5-HT_{1A} receptor-dependent responses more in females. Eur. J. Pharmacol. 164:435–443; 1989.
14. Insel, T. R.; Winslow, J. T. Rat pup ultrasonic vocalizations: An ethological relevant behaviour responsive to anxiolytics. In: Olivier, B.; Mos, J.; Slangen, J. L., eds. Animal models in psychopharmacology. Basel: Birkhäuser Verlag; 1991:15–36.
15. Kellar, K. J.; Hulihan-Giblin, B. A.; Mulroney, S. E.; Lumpkin, M. D.; Flores, C. M. Stimulation of serotonin1A receptors increases release of prolactin in the rat. Neuropharmacology 31:643–647; 1992.
16. Kennett, G. A.; Dickinson, S. L.; Curzon, G. Enhancement of some 5-HT dependent behavioural responses following repeated immobilization in rats. Brain Res. 330:253–263; 1985.
17. Molewijk, H. E.; van der Poel, A. M.; Mos, J.; van der Heyden, J. A. M.; Olivier, B. Conditioned ultrasonic distress vocalizations in adult male rats as a behavioural paradigm for screening anti-panic drugs. Psychopharmacology (Berlin) 117:32–40; 1995.
18. Mundey, M. K.; Fletcher, A.; Marsden, C. A. Effect of the putative 5-HT_{1A} antagonist WAY100635 on 5-HT neuronal firing in the guinea-pig dorsal raphé nucleus. Br. J. Pharmacol. 112:93P; 1994.
19. Olivier, B.; Mos, J.; Molewijk, E.; Zethof, T.; Van der Poel, G. Preclinical evidence for the role of serotonin receptors in anxiety. In: Vanhoutte, P. M.; Paoletti, R.; Brunell, N.; Jackson, A. S., eds. Serotonin: From cell biology to pharmacology and therapeutics. The Netherlands: Kluwer Academic Publishers; 1993: 205–210.
20. Park, S. B. G.; Owen, P. J. Effect of pindolol on the prolactin response to d-fenfluramine. Psychopharmacology (Berlin) 118: 471–474; 1995.
21. Saphier, D.; Welch, J. E. 5-HT_3 receptor activation in the rat increases adrenocortical secretion at the level of the central nervous system. Neurosci. Res. Commun. 14:167–173, 1994.

22. Shepherd, J. K.; Conbain, M. R.; Fletcher, A.; Marsden, C. A. Ferret territorial behaviour is sensitive to diazepam and antagonist action at the 5-HT_{1A} receptor. Behav. Pharmacol. 5S:82; 1994.
23. Stanhope, K. J.; Dourish, C. T. Effects of 5-HT_{1A} receptor agonists and silent 5-HT_{1A} receptor antagonist in the conditioned emotional response test in rats. Behav. Pharmacol. 5S:32; 1994.
24. Urban, J. H.; Van de Kar, L. D.; Lorens, S. A.; Bethea, C. L. Effect of the anxiolytic drug buspirone on prolactin and corticosterone secretion in stressed and unstressed rats. Pharmacol. Biochem. Behav. 25:457–462; 1986.
25. Van Dijken, H. H.; deGoeij, D. C. E.; Sutanto, W.; de Kloet, E. R.; Tilders, F. J. H. Short inescapable stress produces long-lasting changes in brain–pituitary–adrenal axis of adult male rats. Neuroendocrinology 58:57–64; 1993.