Pharmacology Biochemistry and Behavior, Vol. 60, No. 1, pp. 27–32, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(97)00482-6

Modification of the Anxiolytic Action of 5-HT_{1A} Compounds by GABA–Benzodiazepine Agents in Rats

A. FERNÁNDEZ-GUASTI AND C. LÓPEZ-RUBALCAVA

Sección de Terapéutica Experimental, Departamento de Farmacología y Toxicología, CINVESTAV, and División de Investigaciones en Neurociencias, Instituto Mexicano de Psiquiatría, México D.F., México

Received 7 February 1997; Revised 23 June 1997; Accepted 28 July 1997

FERNÁNDEZ-GUASTI, A. AND C. LÓPEZ-RUBALCAVA. *Modification of the anxiolytic action of 5-HT_{1A} compounds by GABA–benzodiazepine agents in rats.* PHARMACOL BIOCHEM BEHAV **60**(1) 27–32, 1998.—The general purpose of the present study was to analyze the possible interactions between the GABA–benzodiazepine and the serotonin systems in the mediation of the antianxiety actions of 5-HT_{1A} compounds. The anxiolytic effect of buspirone (5 mg/kg), ipsapirone (5 mg/kg), indorenate (5 mg/kg), and 8-OH-DPAT (0.5 mg/kg) was established in the rat burying behavior test. Flumazenil (5 mg/kg), but not bicuculline (2.5 mg/kg), effectively counteracted the reduction in burying behavior produced by buspirone, ipsapirone, and 8-OH-DPAT. These same 5-HT_{1A} compounds, at subthreshold doses, produced an important reduction in burying behavior when combined with diazepam (0.25 mg/kg). The effect of indorenate was not altered by any of the antagonists and, when combined with diazepam it produced large increases in burying behavior latency. Only buspirone alone and in combination with bicuculline or flumazenil impaired motor coordination as tested in the rota rod. Data are discussed on the bases of the interaction between the GABAergic and serotonergic systems, stressing species differences and variations due to the animal model of anxiety. © 1998 Elsevier Science Inc.

Rat burying behavior 5-HT_{1A} ligands Flumazenil bicuculline GABA–serotonin interactions

IN the modulation of anxiety, several neurotransmitter systems have been involved. Among them, serotonin seems particularly interesting, because various compounds acting at 5-HT_{1A} receptors possess antianxiety properties both clinically (20,21,33) and in various animal models of anxiety (4,15, 22). The 5-HT_{1A} compounds, buspirone, ipsapirone, indorenate, and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) share with benzodiazepines the ability to reduce anxiety (10,29), but lack some of their adverse effects such as tolerance, dependence, and motor disruptions (21,24,33).

At present, it is well established that benzodiazepines exert their anxiolytic actions through the GABAergic system [cf. (37)]. However, preclinical data show that diazepam also affects the extracellular levels of serotonin in the ventral hippocampus and that such changes correlate with a reduction in anxiety (49). Because benzodiazepines inhibit the firing of serotonergic neurons in the dorsal raphe nucleus (40,47) and decrease 5-HT turnover (7,28), it was suggested that these compounds might produce their anxiolytic effects acting upon serotonergic neurons. However, several evidences argue against this idea. Possibly the most conclusive data were reported by Thiebot et al. (45), who showed that even after lesioning the serotonergic system the benzodiazepines are still able to produce their anxiolytic actions. In spite of all these facts, it has been recently proposed (17) that benzodiazepines might directly interact with the serotonergic system (possibly through the 5-HT_{1B} receptor) to produce some of their antianxiety actions.

Several results indicate a possible GABA–benzodiazepine mediation of the anxiolytic action of various serotonergic drugs. Thus, Söderpalm and Engel, in 1989 (43), showed that both the benzodiazepine receptor antagonist, flumazenil, and the $GABA_A$ receptor antagonist, bicuculline, counteracted the *p*-chlorophenylalanine (*p*-CPA)-induced anticonflict effect at doses not affecting the behavior per se—10.0 and 2.0 mg/kg, respectively. Additionally, we demonstrated that flu-

Requests for reprints should be addressed to Dr. Alonso Fernández-Guasti, Sección de Terapéutica Experimental, Departamento de Farmacología y Toxicología, Centro de Investigación y de Estudios, Avanzados, Ap. Postal 22026, México 14000, D.F., México.

mazenil counteracted the anxiolytic action of ipsapirone, indorenate, and 8-OH-DPAT (29) in mice using an anxiety paradigm designed for this species: the black and white transition test (8). Recently, important species (2,12) and anxiety paradigms (4,31) differences, particularly for the action of 5-HT_{1A} drugs, have been addressed. Thus, it has been shown that buspirone exhibits anxiolytic actions in rats, but not in mice and hamsters (10–14). Moreover, it seems that in the anxiolytic action of these 5-HT_{1A} agonists, the noradrenergic system is involved in rats, but not in mice (30). In relation to the differences in the anxiety paradigms, several authors have found anxiolysis (10,31), no effect (27,34), and even anxiogenic activity (9,26) of the 5-HT_{1A} compounds, depending upon the animal model of anxiety used.

On these bases, the purpose of the present study was to analyze the possible interaction between the serotonergic and GABAergic neurotransmitter systems in the anxiolytic action of buspirone, ipsapirone, indorenate, and 8-OH-DPAT in rats. Such interaction was studied by attempting to counteract the anxiolytic effects of these drugs with either the selective benzodiazepine antagonist, flumazenil (25), or the competitive GABA antagonist, bicuculline. A putative synergistic effect of low doses of the serotonergic anxiolytics with diazepam was also investigated. In the present study anxiety levels were determined using the rat defensive burying behavior test. This test was chosen on the basis of the important advantages it offers such as the specificity for detecting anxiolytic actions produced either by pharmacological (46) or physiological (11,38) manipulations, the simple experimental procedure (39), our laboratory's ample knowledge and experience in this test using serotonergic compounds (10,13,30–32), and the possibility to compare present results with previous data obtained in the same animal paradigm. A motor coordination test was conducted in parallel to detect possible unspecific drug actions.

METHOD

Animals

Male adult Wistar rats (285–300 g b.wt.) were used in this study. All animals were individually caged in a room under inverted and controlled light dark cycle conditions (12 L:12 D—lights on at 2200 h). Subjects had ad lib access to water and Purina rat chow throughout the experiment.

Drugs

The drugs used in the present study were ipsapirone (Miles Pharmaceutical Division, West Haven, CT), indorenate (Department of Pharmacology, CINVESTAV, México City, México), buspirone (Mead Johnson, México City, México), 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT, Biochemical Research, Natick, MA), flumazenil (Hoffmann–LaRoche, México City, México), bicuculline (Sigma Chemicals, St Louis, MO), and diazepam (Hoffmann–LaRoche, México City, México). All serotonergic compounds were dissolved in physiological saline and IP injected. Flumazenil was dissolved in a drop of Tween 80 and thereafter in distilled water. Diazepam was dissolved in propylene glycol 40%. Bicuculline was dissolved in a drop of HCl (0.1 N) and saline; NaOH (0.1 N) was used to adjust pH to 5.0.

Procedure

The effect of flumazenil and bicuculline on the anxiolytic action of 5-HT_{1A} compounds in the burying behavior test was studied. In these series of experiments animals were injected with anxiolytic doses of buspirone (5 mg/kg, −30 min), ipsapirone (5 mg/kg, −30 min), indorenate (5 mg/kg, −90 min), and 8-OH-DPAT (0.5 mg/kg, −20 min). For each of these treatments, flumazenil (5 mg/kg) or bicuculline (2.5 mg/kg) was administered 5 min before the 5-HT_{1A} drug. The doses were selected on the basis of previous reports (11,29,43).

In a subsequent experiment, the effect of suboptimal doses of diazepam plus the 5-HT_{1A} anxiolytics on burying behavior was analyzed. To this purpose animals received subthreshold doses of buspirone (2.5 mg/kg, −30 min), ipsapirone (2.5 mg/kg, −30 min), indorenate (2.5 mg/kg, −90 min), 8-OH-DPAT (0.125 mg/kg, −20 min), or diazepam (0.25 mg/kg, −30 min). In the combined trials, diazepam and the 5-HT_{1A} compounds were simultaneously injected and the observations performed after the respective latency for each 5-HT_{1A} drug. Doses of the 5-HT_{1A} compounds (10,12,32) and of diazepam (29) were selected based on their inability to produce anxiolytic responses. Control groups receiving the respective vehicles were included. In all cases a minimum of 10 animals were tested.

Anxiety Test

The burying behavior test has been previously described in detail and validated as a useful and selective model for establishing experimental anxiety (39). Briefly, this paradigm consists of an acrylic cage (27 × 16 × 23 cm) with a prod (7 cm long) emerging from one of its walls (2 cm above the bedding material) and the floor covered with fine sawdust. This experimental cage possesses the same measurements as the home cage; however, animals were never before the test, presented to the prod. Through the prod the animal receives an electric shock of 0.3 mA. The electric source consisted of a constant current shocker (LaFayette Instruments Co., model 5806). During the test the animal was placed in the cage and its behavior recorded during a 10-min period. Each time the animal touched the prod it received an electric shock and typically displayed the burying behavior that consists of a series of rapid and alternating movements of its forepaws, moving and pushing a pile of bedding material over the aversive stimulus: the electrified prod. Two main parameters were registered: the burying behavior latency (time between the first shock and the burying behavior display), and the cumulative burying behavior (cumulative time that the animal spends burying the prod during a 10-min test). We have observed in our laboratory that around 17% of the rats spontaneously do not display the burying behavior (unpublished observations). Therefore, the animals showing burying behavior values of 0 and latencies of 600 s were eliminated from this study.

It has been considered that burying behavior latency inversely reflects the animals' reactivity, while the cumulative burying behavior directly denotes the experimental anxiety levels (39,46).

Motor Coordination Test

In addition to the anxiety trial, a motor coordination test was performed in a treadmill apparatus (rotarod). Briefly, this test consisted in placing the animals upon a cylinder (diameter 7 cm) rotating at a speed of 11 rpm. These animals were trained to walk on the cylinder on three previous consecutive sessions, and on the fourth the animals received the pharmacological treatments. The number of falls during a 5-min period was counted. After a fall the animal was immediately returned to the cylinder.

Statistics

The parameters registered in the burying behavior test were compared using the Kruskal–Wallis analysis of variance followed by the Mann–Whitney *U*-test. In the motor coordination test, the number of falls in the experimental day were compared to those in the previous session (data from the day before) by means of the Wilcoxon *t*-test (42).

RESULTS

Figure 1 shows the reduction in burying behavior, interpreted as an anxiolytic action, produced by buspirone, ipsapirone, indorenate, and 8-OH-DPAT (dark bars). Administration of flumazenil effectively reversed the reduction in burying behavior induced by buspirone, ipsapirone, and 8-OH-DPAT, but not that of indorenate. Conversely, bicuculline did not modify the anxiolytic effect of the serotonergic drugs, except for 8-OH-DPAT. In this case, a partial antagonism was found.

Figure 2 shows the burying behavior latency after the same treatments showed in Fig. 1. Except for indorenate, no other 5-HT$_{1A}$ compound alone or in combination with flumazenil or bicuculline, affected this parameter. Clearly, the combined treatment of flumazenil or bicuculline with indorenate significantly prolonged the burying behavior latency.

The interactions between these 5-HT$_{1A}$ compounds and diazepam on the cumulative burying behavior are shown in Fig. 3. Low doses of buspirone, ipsapirone, indorenate, 8-OH-DPAT (dark bars), and diazepam (dashed bars) did not affect the burying behavior response. The combined treatment of diazepam with low doses of all serotonergic compounds tested, reduced this behavior. Except for indorenate, which combined with diazepam augmented the burying behavior latency, no other treatment affected this parameter (Fig. 4).

Table 1 shows the effect of the 5-HT$_{1A}$ compounds 8-OH-DPAT, ipsapirone, buspirone, and indorenate alone and in combination with the GABA–benzodiazepine compounds on motor coordination. In this case, buspirone decreased motor coordination per se, and in combination with flumazenil or bicuculline. However, when combined with diazepam, the effect of buspirone disappeared. No other treatment altered motor coordination.

DISCUSSION

In the present study, we analyzed the interactions between the serotonergic and the GABA–benzodiazepine systems in the mediation of the anxiolytic actions of the 5-HT$_{1A}$ compounds buspirone, ipsapirone, 8-OH-DPAT, and indorenate in the rat.

In the first part of this work, the experiments show that the GABA–benzodiazepine antagonist, flumazenil, is able to prevent the anxiolytic action of ipsapirone, buspirone, and 8-OH-DPAT, but not that of indorenate. These results are in line with previous data showing that flumazenil effectively blocks the anxiolytic actions of serotonergic drugs in other anxiety paradigms: the black and white transitions test (29) and the modified Vogel conflict paradigm (43). By contrast, the GABA antagonist bicuculline fails to block the anxiolytic action of the serotonergic drugs. Such lack of action cannot be interpreted on the basis of the dose level used, because similar concentrations of bicuculline have proven to be effective in antagonizing pharmacological (43) and physiological (11) anxiolytic effects. Thus, these data suggest that the benzodiazepine, rather than the GABAergic, receptor is involved in the anxiolysis observed with 5-HT$_{1A}$ ligands.

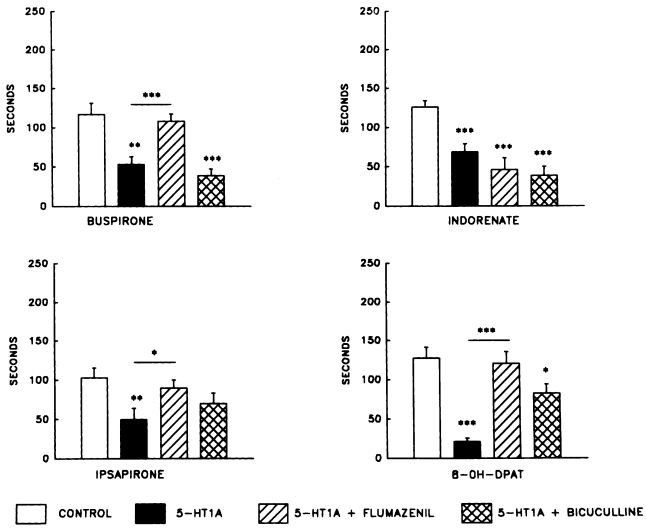

FIG. 1. Effect of flumazenil or bicuculline (dashed bars) on the reduction of burying behavior produced by various 5-HT$_{1A}$ compounds (dark bars). The figure shows mean ± SE cumulative burying behavior expressed in seconds. Asterisks over columns represent comparisons vs. control vehicle-treated group (clear bars). Other comparisons are shown by brackets. Mann–Whitney *U*-test, $*p < 0.05$; $**p < 0.02$; $***p < 0.01$.

To date, it is well established that the 5-HT$_{1A}$ compounds 8-OH-DPAT, indorenate, ipsapirone, and buspirone neither stimulate nor inhibit [^{3}H]benzodiazepine binding, do not affect the influence of GABA on benzodiazepine binding, and do not interfere with GABA binding or uptake (44). Further-

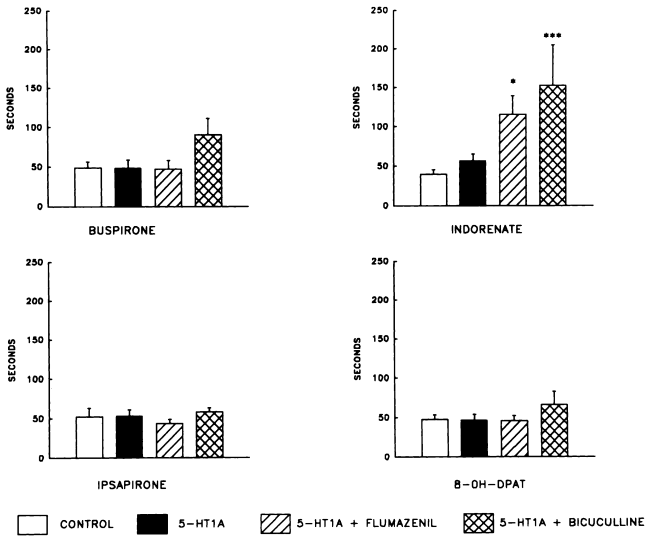

FIG. 2. Effect of various 5-HT$_{1A}$ compounds administered alone (dark bars) or in combination with either flumazenil or bicuculline (dashed bars). The figure shows mean ± SE burying behavior latency expressed in seconds. Asterisks over columns represent comparisons vs. control vehicle-treated group (clear bars). Mann–Whitney *U*-test, $*p < 0.05$; $***p < 0.01$.

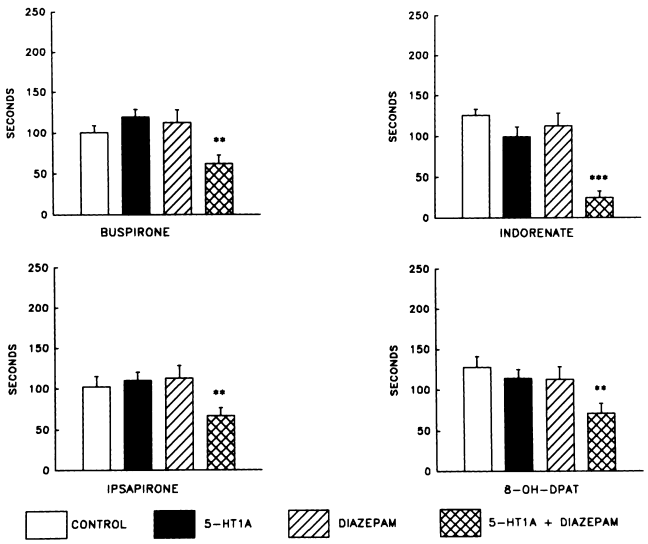

FIG. 3. Potentiative actions of combining subthreshold doses of various 5-HT_{1A} compounds (dark bars) with diazepam (dashed bars) on the cumulative burying behavior. The figure shows mean ± SE cumulative burying behavior expressed in seconds. Asterisks over columns represent comparisons vs. control vehicle-treated group (clear bars). Mann–Whitney *U*-test, $^{**}p < 0.02$; $^{***}p < 0.01$.

more, buspirone, by contrast with diazepam, does not potentiate GABA inhibition at benzodiazepine-linked GABA receptor sites (41), or does not potentiate or block the effects of iontophoretically applied GABA (48). Additionally, flumazenil is well known to be a specific antagonist of benzodiazepine receptors that lacks serotonergic activity (25). These data indicate that serotonergic compounds and benzodiazepines do

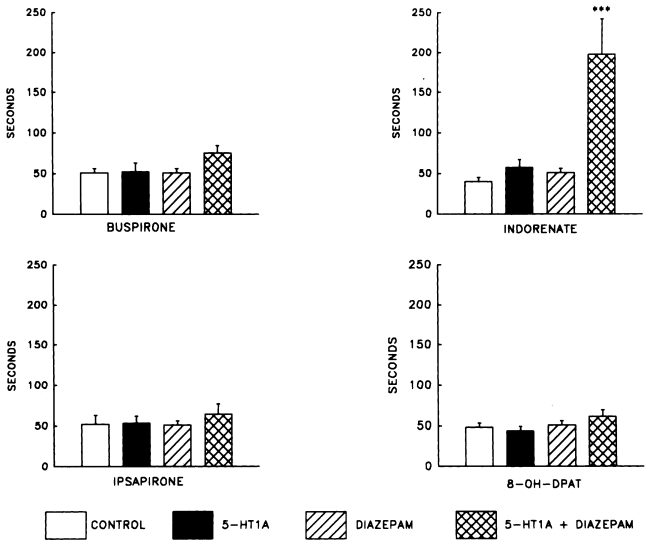

FIG. 4. Effect of subthreshold doses of various 5-HT_{1A} compounds administered alone (dark bars) or in combination with diazepam (dashed bars) on the burying behavior latency. The figure shows mean ± SE burying behavior latency expressed in seconds. Asterisks over columns represent comparisons vs. control vehicle-treated group (clear bars). Mann–Whitney *U*-test, $^{***}p < 0.01$.

TABLE 1

EFFECT OF THE 5-HT_{1A} LIGANDS ALONE AND IN COMBINATION WITH THE GABA/BENZODIAZEPINE COMPOUNDS ON THE MOTOR COORDINATION TEST

Compound	C, T	+ Flumazenil C, T	+ Bicuculline C, T	+ Diazepam C, T
8-OH-DPAT (0.5 mg/kg)	1, 3	1, 2	2, 2	1, 2
Indorenate (5.0 mg/kg)	0, 0	1, 1	1, 1	1, 1
Ipsapirone (5.0 mg/kg)	0, 1	1, 1	1, 2	1, 1
Buspirone (5.0 mg/kg)	0, 6*	1, 5*	1, 6*	1, 1

Table shows median numbers of falls: C—control; T—treated; Wilcoxon's *t*-test: $^{*}p < 0.001$.

not share the same receptor. However, because flumazenil blocked the antianxiety actions of the 5-HT_{1A} compounds, a possible interaction between the GABAergic and the serotonergic neurotransmitter systems can be proposed. Indeed, we and others (29,43) have previously posed the nature of such interaction.

Nevertheless, there are also reports indicating that flumazenil is unable to prevent the anxiolytic actions of the serotonergic compounds in other anxiety paradigms. For example, it has been demonstrated that the increase in punished responding, produced by buspirone (2) or ipsapirone (50), is not affected by this benzodiazepine antagonist. The reasons underlying these discrepancies, at present, remain unclear. The main differences between these studies and present data are the animal models used (conflict tests), and in one case, a different species (pigeons). Previous studies have reported that both factors may importantly influence the results observed [cf. (4,15,22)]. Thus, variations in the anxiolytic profile of these 5-HT_{1A} compounds among mice, rats, hamsters, and pigeons have been shown (2,10–14).

In the second part of our work, a reduction in burying behavior after the combined administration of subthreshold doses of diazepam and the 5-HT_{1A} anxiolytics was observed. These data agree with clinical studies showing that buspirone and diazepam potentiate in their anxiolytic action, and that small doses of buspirone do not cause any additional decrement in the psychomotor performance induced by diazepam (33). It has been shown that coadministration of buspirone and diazepam does not alter the disposition of these agents or their demethylated metabolites (19). Therefore, the potentiation observed after the combined administration of these drugs could not be explained on the bases of changes in pharmacokinetic parameters, suggesting instead a central interaction.

Data from physical dependence to benzodiazepines also reveal interactions between buspirone and diazepam. Thus, Mizoguchi et al. (35) showed that coadministration of buspirone, but not mianserin (5-HT_{1C} antagonist) or ketanserin (5-HT_2 antagonist), with diazepam potentiated the hypersensitivity to the anxiogenic betacarboline, FG 7142, following chronic treatment with diazepam. This potentiation was not ascribable to pharmacokinetic interactions between diazepam and buspirone, suggesting that the coinjection of buspirone and diazepam may potentiate the development of physical dependence on diazepam and that both drugs may be interacting

in this effect. Moreover, Andrews and File (1,16) have demonstrated that buspirone significantly reverses the anxiogenic effects of diazepam withdrawal, also suggesting that 5-HT_{1A} receptors may be partially involved in the development of physical dependence to diazepam.

Taken together, from these data, it can be concluded that both neurotransmitter systems (GABA and 5-HT) interact in the mediation of the anxiolytic action of the 5-HT_{1A} compounds. The nature of such interaction is yet unknown; however, possible models including connections between serotonergic and GABAergic neurons have been previously proposed (29,43). Furthermore, several anatomical studies show that these two neurotransmitter systems are associated in brain areas related with the modulation of anxiety (18,23,36).

Present results also show that indorenate follows a dissimilar pharmacological profile from that observed for the other 5-HT_{1A} compounds, i.e., its action is neither potentiated by diazepam nor inhibited by flumazenil. The reasons underlying these differences may include the particular pharmacological characteristics of indorenate compared with the other 5-HT_{1A} compounds, such as the lack of noradrenergic and 5-HT_2 activity, as well as, with exception of 8-OH-DPAT, full agonism at both pre- and postsynaptic 5-HT_{1A} receptors (3,5,6,29). Interestingly, the only treatments that prolonged the burying behavior latency involved indorenate combined either with diazepam, flumazenil, or bicuculline. These results suggest an impaired reactivity towards the aversive stimulus (46) and could denote motor deficiencies not affecting, however, motor coordination as tested in the rotarod.

As previously reported (32), among all treatments included in this study only buspirone alone and in combination with flumazenil or bicuculline seems to affect the motor coordination of the rats. However, it is important to mention that the animals "jump off" the cylinder rather than fall down from the rotarod, a behavior that cannot be considered as an evidence of motor coordination impairment. Interestingly, diazepam concomitantly administered with buspirone prevents this particular behavior. Further experiments employing other motor coordination tests should be undertaken to fully characterize buspirone actions on motor activity.

The present series of experiments showing (a) a complete blockade of the anxiolytic action of buspirone, ipsapirone, and 8-OH-DPAT after flumazenil injection; and (b) a reduction in burying behavior after combining subthreshold doses of the serotonergic drugs with diazepam, suggest an interaction between the serotonergic and GABA–benzodiazepine systems in the anxiolytic action of 5-HT_{1A} compounds.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Facundo Moreno for technical assistance, Mr. Víctor Flores for animal caring, and Dra. Gabriela Rodrìguez Manzo for language checking. The "Consejo Nacional de Ciencia y Tecnología, CONACyT" is acknowledged for partially supporting this investigation (Grant to A.F-G. No. 2338P).

REFERENCES

1. Andrews, N.; File, S. E.: Increased 5-HT release mediates the anxiogenic response during benzodiazepine withdrawal: A review of supporting neurochemical and behavioral evidence. Psychopharmacology (Berlin) 112:21–25; 1993.
2. Barrett, J. E.; Witkin, J. M.; Mansbach, R. S.; Skolnick, P.; Weissman, B. A.: Behavioral studies with anxiolytic drugs. III. Antipunishment actions of buspirone in the pigeon do not involve benzodiazepine receptor mechanisms. J. Pharmacol. Exp. Ther. 238:1009–1013; 1986.
3. Benítez-King, G.; Antón-Tay, F.; Hong, E.: Characterization of indorenate effects on brain monoamine metabolism. Drug Dev. Res. 23:433–437; 1991.
4. Broekkamp, C. L. E.; Berendsen, H. H. G.; Jenck, F.; Van Delft, A. M. L.: Animal models for anxiety and response to serotonergic drugs. Psychopathology 22(Suppl. 1):2–12; 1989.
5. Castillo, C.; Ibarra, M.; Márquez, J. A.; Villalobos-Molina, R.; Hong, E.: Pharmacological evidence for interactions between 5-HT_{1A} receptor agonists and subtypes of alpha1-adrenoceptors on rabbit aorta. Eur. J. Pharmacol. 241:141–148; 1993.
6. Castillo, C.; Ibarra, M.; Terrón, J. A.; Villalón, C. M.; Hong, E.: Direct effects of indorenate and 8-OH-DPAT on the blood pressure of pithed rats. Drug Dev. Res. 33:20–25; 1994.
7. Chase, T. N.; Katz, R. I.; Kopin, I. J.: Effect of diazepam on fate of intracisternally injected serotonin-c14. Neuropharmacology 9:103–108; 1970.
8. Crawley, J.; Goodwin, F.: Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol. Biochem. Behav. 13:167–170; 1980.
9. Davis, M.; Cassella, J. V.; Kehne, J. H.: Serotonin does not mediate anxiolytic effects of buspirone in the fear-potentiated startle paradigm: Comparison with 8-OH-DPAT and ipsapirone. Psychopharmacology (Berlin) 94:14–20; 1988.
10. Fernández-Guasti, A; Hong, E.: Antianxiety effect of various putative S1 receptor agonists on the conditioned defensive-burying paradigm. In: Evans, P.; Archer, A. R.; Archer, T., eds. Behavioral pharmacology of 5-HT. Hillsdale, NJ: Lawrence Erlbaum; 1989:377–382.
11. Fernández-Guasti, A.; Saldívar, A.: Participation of the GABA-benzodiazepine system in the inhibition of defensive burying produced by ejaculation. Behav. Pharmacol. 1:429–436, 1990.
12. Fernández-Guasti, A.; Hong, E.; López-Rubalcava, C.: Species differences in the mechanism through which the serotonergic agonists indorenate and ipsapirone produce their anxiolytic action. Psychopharmacology (Berlin) 107:61–68; 1992.
13. Fernandez-Guasti, A.; López-Rubalcava, C.; Pérez-Urizar, J.; Castañeda-Hernandez, G.: Evidence for a postsynaptic action of the serotonergic anxiolytics ipsapirone, indorenate and buspirone. Brain Res. Bull. 28:497–501; 1992.
14. Fernández-Guasti, A.; López-Rubalcava, C.: Action of ipsapirone and 8-OH-DPAT on exploratory behavior in hamsters (*Mesocricetus auratus*): Effects of antagonists and p-CPA. Pharmacol. Biochem. Behav. 50:375–382; 1995.
15. File, S. E.: Animal models for predicting efficacy of anxiolytic drugs: Social behavior. Neuropsychobiology 13:55–62; 1985.
16. File, S. E.; Andrews, N.: Low but not high doses of buspirone reduce the anxiogenic effects of diazepam withdrawal. Psychopharmacology (Berlin) 105:578–582; 1991.
17. Frances, H.; Khidichian, F.; Monier, C.: Benzodiazepines impair a behavioral effect induced by stimulation of 5-HT_{1B} receptors. Pharmacol. Biochem. Behav. 35:841–845; 1990.
18. Gallager, D. W.: Benzodiazepines: Potentiation of a GABA inhibitory response in the dorsal raphe nucleus. Eur. J. Pharmacol. 49:133–143; 1978.
19. Gammans, R. E.; Mayol, R. F.; LaBudde, J. A.: Metabolism and disposition of buspirone. Am. J. Med. 80:41–51; 1986.
20. Goa, K.; Ward, A.: Buspirone: A preliminary review of its pharmacological properties and therapeutic efficacy as an anxiolytic. Drugs 32:114–129; 1986.
21. Goldberg, H. L.; Finnerty, R. J.: The comparative efficacy of buspirone and diazepam in the treatment of anxiety. Am. J. Psychiatry 136:1184–1187; 1979.
22. Handley, S. L.: Serotonin in animal models of anxiety: The importance of stimulus and response. In: Idzikowski, C.; Cowen, P. J., eds. Serotonin, sleep and mental disorders. Petersfield: Wrightson Biomedical; 1991:89–115.
23. Harandi, M.; Aguera, M.; Gamrani, H.; Didier, M.; Maitre, M.;

Calas, A.; Belin, M. F.: Gamma-aminobutyric acid and 5-hydroxytryptamine. Interrelationship in the rat nucleus dorsalis: Combination of radiographic and immunocytochemical techniques at light and electron microscope levels. Neuroscience 21:237–251; 1987.
24. Hong, E.; Villalón, C.: External carotid vasodilation induced by serotonin and indorenate. Proc. West. Pharmacol. Soc. 31:99; 1988.
25. Hunkeler, W. H.; Mohler, L.; Pieri, P.; Polc, E. P.; Bonetti, E. P.; Cumin, R.; Schaffner, R.; Haefely, W.: Selective antagonists of benzodiazepines. Nature 290:514–515; 1981.
26. Jenck, F.; Broekkamp, C. L.; Van Delft, A. M. L.: Opposite control mediated by central 5-HT_{1A} and non 5-HT_{1A} (5-HT_{1B} or 5-HT_{1C}) receptors on periaqueductal gray aversion. Eur. J. Pharmacol. 161:219–221; 1989.
27. Kilts, C. D.; Commissaris, R. L.; Cordon, J. J.; Rech, R. H.: Lack of central 5-hydroxytryptamine influence on the anticonflict activity of diazepam. Psychopharmacology (Berlin) 78:156–164; 1982.
28. Lidbrink, P.; Carrodi, H.; Fuxe, K.; Olson, L.: The effect of benzodiazepines, moprobamate and barbiturates on central monoamines neurones. In: Garattini, S.; Mussini, E.; Randal, L. O., eds. The benzodiazepines. New York: Raven Press; 1972:203–224.
29. López-Rubalcava, C.; Saldívar, A.; Fernández-Guasti, A.: Interaction of GABA and serotonin in the anxiolytic action of diazepam and serotonergic anxiolytics. Pharmacol. Biochem. Behav. 43:433–440; 1992.
30. López-Rubalcava, C.; Fernández-Guasti, A.: Noradrenaline–serotonin interactions in the anxiolytic effects of 5-HT_{1A} agonists. Behav. Pharmacol. 5:42–51; 1994.
31. López-Rubalcava, C.: Pre- or postsynaptic activity of 5-HT_{1A} compounds in mice depends on the anxiety paradigm. Pharmacol. Biochem. Behav. 54:677–686; 1996.
32. López-Rubalcava, C.; Fernández-Guasti, A; Urbá-Holmgren, R.: Age-dependent differences in the rat's conditioned defensive burying behavior: Effect of 5-HT_{1A} compounds. Dev. Psychobiol. 29:157–169; 1996.
33. Mattila, M.; Seppala, T.; Mattila, M. J.: Combined effects of buspirone and diazepam on objective and subjective tests of performance in healthy volunteers. Clin. Pharmacol. Ther. 40:620–626; 1986.
34. Meert, T. F.; Colpaert, F. C.: The shock probe conflict procedure: A new assay responsive to benzodiazepines, barbiturates and related compounds. Psychopharmacology (Berlin) 88:445–450; 1986.
35. Mizoguchi, H.; Suzuki, T.; Misawa, M.: Effects of serotonergic anxiolytics on physical dependence on diazepam in mice. Neurosci. Lett. 160:41–44; 1993.
36. Nishikawa, T; Scatton, B.: Neuroanatomical site of the inhibitory influence of anxiolytic drugs on central serotonergic transmission. Brain Res. 377:123–132; 1986.
37. Olsen, R. W.; Venter, J. C.: Benzodiazepine-GABA receptors and chloride channels: Structural and functional properties. New York: Alan R. Liss; 1985.
38. Picazo, O.; Fernández-Guasti, A.: Changes in experimental anxiety during pregnancy and lactation. Physiol. Behav. 54:295–299; 1993.
39. Pinel, J. P. J.; Treit, D.: Burying as a defensive response in rats. J. Comp. Physiol. Psychol. 92:708–712; 1978.
40. Pratt, J.; Jenner, P.; Reynolds, E. H.; Marsden, C. D.: Clonazepam induces decrease serotonin activity in mouse brain. Neuropharmacology 18:791–799; 1979.
41. Sanghera, M. K.; German, D. C.: The effects of benzodiazepine and nonbenzodiazepine anxiolytics on locus coeruleus unit activity. J. Neural. Transm. 57:267–279; 1983.
42. Siegel, S.: Non parametric statistics for the behavioral sciences. New York: McGraw Hill; 1956.
43. Söderpalm, B.; Engel, J. A.: Does the p-CPA induced anticonflict effect involve activation of the $GABA_A$/benzodiazepine chloride ionophore receptor complex? J. Neural Transm. 76:145–153; 1989.
44. Taylor, D. P.; Riblet, L. A.; Stanton, H. C.; Eison, A. S.; Eison, M. S.: Dopamine and antianxiety activity. Pharmacol. Biochem. Behav. 17(Suppl 1):25–35; 1982.
45. Thièbot, M. H.: Are serotonergic neurons involved in the control of anxiety and in the anxiolytic activity of benzodiazepines? Pharmacol. Biochem. Behav. 24:1471–1477; 1986.
46. Treit, D.: Animal models for the study of anti-anxiety agents. A review. Neurosci. Biobehav. Rev. 9:203–222; 1985.
47. Trulson, M. E.; Preusler, D. W.; Howell, G. A.; Frederickson, C. J.: Raphe unit activity in freely moving cats: Effects of benzodiazepines. Neuropharmacology 21:1050–1082; 1982.
48. Van der Maelen, C. P.; Matheson, G. K.; Wilderman, R. C.; Patterson, L. A.: Inhibition of serotonergic dorsal raphe neurons by systemic and iontophoretic administration of buspirone, a nonbenzodiazepine anxiolytic drug. Eur. J. Pharmacol. 129:123–130; 1986.
49. Wright, I. K.; Upton, N.; Marsden, C. A.: Effect of established and putative anxiolytics on extracellular 5-HT and 5-HIAA in the ventral hippocampus of rats during behavior on the elevated X-maze. Psychopharmacology (Berlin) 109:338–346; 1992.
50. Yamamoto, T.; Shibata, S.; Teshima, K.; Inoue, Y.; Ushio, M.; Tominaga, K.; Ohno, M.; Watanabe, S.; Ueki, S.: Behavioral pharmacological and electroencephalographic effects of the 5-HT_{1A} partial agonist ipsapirone. Nippon Yakurigaku. Zasshi. 98:41–52; 1991.