Pharmacology Biochemistry and Behavior, Vol. 46, pp. 569–573, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Modification of the Anxiolytic Effects of 5-HT_{1A} Agonists by Shock Intensity

ALFREDO MENESES[1] AND ENRIQUE HONG

Sección de Terapéutica Experimental, Departamento de Farmacología y Toxicología, CINVESTAV-IPN. Ap. Postal 22026, México, D.F., 14000 México

Recieved 11 December 1992

MENESES, A. AND E. HONG. *Modification of the anxiolytic effects of 5-HT_{1A} agonists by shock intensity.* PHARMACOL BIOCHEM BEHAV 46(3) 569–573, 1993.—Contradictory evidence exists concerning the anxiolytic effects of 5-HT_{1A} agonists in the conflict test. In the present work, a modification of the Vogel conflict model was used to assess different doses of diazepam (0.1–5.6 mg/kg), ipsapirone (1.0–17.8 mg/kg), buspirone (1.7–17.8 mg/kg), and indorenate (0.56–17.8 mg/kg) in rats receiving two different electric shock intensities (0.16 and 0.32 mA). The results show that the three 5-HT_{1A} agonists had a smaller anticonflict effect than diazepam. The anticonflict effect with each compound was of a greater magnitude at 0.16 mA intensity than at 0.32 mA. This study shows that, using different electric shock intensities, compounds produce a differential effect: the anticonflict effects were more pronounced with the lower electric shock intensity than with the higher intensity. The present results suggest that the use of different shock intensities can play distinct roles over the drug's effect in the conflict test.

Serotonin 5-HT_{1A} agonists Conflict test Shock intensity Diazepam Ipsapirone Buspirone Indorenate Anxiety Rats

SEVERAL models of conflict have been used in the search for new anxiolytic agents (9,12,29,36). Among these models, the most popular is the test of Geller-Seifter (13). In this paradigm, the increment in the number of suppressed responses or electric shocks received is taken as an index of anxiolytic activity (6). This conflict test has been modified by other investigators [i.e., instead of the lever press response, licking has been used (37)]. Another modification consisted in the individual adjustement of electric shock intensities (30,37). These modifications improve the conflict test, since they decrease the time needed for training and allow the establishment of similar levels of suppressed responses between animals.

The identification and development of specific compounds for serotonin receptors and subtypes of receptor (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_2, 5-HT_3, 5-HT_4) (27) has allowed the study of the role of serotonin in the genesis and therapeutics of anxiety.

Several 5-HT_{1A} agonists have been assessed in different versions of the conflict test with contradictory results. For instance, buspirone has shown anticonflict effects (10,17,18, 23,24,26,28) or no effect (2,16,31); ipsapirone has also produced anticonflict effects (4,15,18,34) or no effects (7). On the other hand, experiments with pigeons and different 5-HT_{1A} agents in the conflict test have yielded consistent anticonflict effects; the reasons for the different findings with pigeons and rats are unknown (15).

The above contrasting results have been discussed in terms of the anxiolytic properties of those agents (12), but little attention has been paid to other factors, such as the animal's behavioral and pharmacological experiences, particularly drug administration in either acute or chronic phases of the behavioral suppression or conflict period, and fixed vs. adjusted intensities.

It has been stated that anxiety is not a unitary phenomenon (36), and hence it could be expected that several training or test conditions, and/or different electric shock intensities (34) could engender different types or levels of anxiety. In the present work, we evaluated the anxiolytic activity of three 5-HT_{1A} agonists (8,20) and diazepam (as an anxiolytic reference drug) in the Vogel conflict test with two fixed electric shock intensities (0.16 or 0.32 mA). The administration of compounds was made during conflict acquisition (acute phase).

METHOD

Animals

We employed naive, male Wistar rats. When the experiment started the animals were 12 weeks old. They were main-

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

tained for 1 week with ad lib food and water in a temperature- and light-controlled room (12L : 12D starting at 7:00 a.m.). Animals were housed in plastic cages with 10 rats per cage. Experiments were conducted during the light phase.

Conflict Behavior

Apparatus. Operant chambers were employed; each chamber was enclosed in a sound-attenuating compartment equipped with a ventilating fan. The inner dimensions of each chamber were 25 × 29 × 25 cm, with a grid floor of stainless steel bars and a drinking bottle containing tap water. Two walls of the chamber were of aluminum and the other two of Plexiglas. The house light was located in the upper left corner of the front aluminum wall, and the module of white noise was located in the opposing corner. At the center of the same wall there was an opening 10 cm to the left and to the right containing a drinking tube attached to a graduated bottle (50 ml). A photocell was used to detect only the licking response. A source of electric programmable shocks (model E13-16) was also employed, with one pole connected to the grid floor and the other pole to the drinking tube. Two electric shock intensities were used (0.16 and 0.32 mA). All recording and control equipment were Coulbourn Instruments (Lehigh Valley, PA).

General Procedure

The experiment consisted of two phases: 1) training to drink from the drinking tube, and 2) test, which consisted of administration of the drug plus delivery of electric shocks.

Training. The animals were maintained for 1 week in the light- and temperature-controlled room with free access to food and water. They were water deprived for 48 h and then each animal was placed in the conditioning chamber and was allowed to drink tap water for 10 min, then the subject was placed in its home cage. In this phase the number of licks were recorded.

Test. After 24 h of water deprivation, drugs were administred and animals were returned to the conditioning chamber. When approaching the drinking tube, animals were allowed to drink until completing 150 licks. Immediately after, electric shock were delivered for 2 s every 5 s. The lenght of the test phase was 10 min. The following parameters were measured: the number of licks during the 2 s of electric shock delivery (conflict period), and the number of licks during the asbence of electric shock (licks not suppressed). It is noteworthy that animals can make several licks per electric shock delivered; therefore, animals received more than one or two electric shocks during the 2 s. The two intensity levels were tested with different doses of each compound and each animal was used only once.

Drugs. Diazepam (0.1–5.6 mg/kg) (Hoffmann-La Roche, México) was prepared in 0.5% methylcellulose. Buspirone (1.7–17.8 mg/kg) (Mead Johnson, México), ipsapirone (1.0–17.8 mg/kg) (Miles Pharmaceutical Division, West Haven, CT), and indorenate (0.56–17.8 mg/kg) (CINVESTAV-IPN, México) were dissolved in a 0.9% saline solution. All drugs were injected intraperitoneally (IP) in a volume of 1 ml/kg 30 min before the test, except indorenate, which was administred 90 min before the test.

Statistical Analysis

The effects of single doses of various drugs on the conflict test performance were examined by one-way ANOVA and post hoc comparison with control by Dunnett's *t*-test. Dose-response curves for each drug were compared using a factorial ANOVA for unpaired samples with two factors to detect the interaction between drug per electric shock intensities. In all statistical comparisons, $p < 0.05$ was used as the criterion for statistical significance.

RESULTS

The analysis of number of electric shocks received for each compound showed that these anticonflict effects were greater at the intensity of 0.16 mA than with 0.32 mA. Diazepam increased the number of electric shocks received (Fig. 1). When the ANOVA test was used, the greatest effect in electric shock intensity was at 0.16 mA in comparison to 0.32 mA, $F(1, 9) = 27.86$, $p < 0.01$, and the results with both intensities were different with respect to the control vehicle group, $F(9, 140) = 8.85$, $p < 0.01$. A further analysis, with Dunnett's *t*-test, showed that diazepam increased the number of electric shocks received in a dose-related fashion. Statistically significant effects were observed with doses from 1.0 to 3.1 mg/kg with both intensities. The number of licks not suppressed were increased between the doses of 0.31 to 10.0 mg/kg.

Ipsapirone increased the number of electric shocks received (Fig. 2). The ANOVA for this compound revealed a significant effect in the number of electric shocks received in relationship to shock intensities, $F(1, 6) = 34.93$, $p < 0.01$, and doses tested, $F(6, 162) = 6.52$, $p < 0.01$. There was also a significant interaction between electric shock intensities and some doses, $F(6, 162) = 5.60$, $p < 0.01$. Dunnett's *t*-tests showed that ipsapirone displayed significant anticonflict effects only with the intensity of 0.16 mA, while it was devoid of activity at 0.32 mA. Licks not suppressed were significantly increased in a dose-dependent fashion only when 0.16 mA intensity was used.

The ANOVA for buspirone (Fig. 3) showed a statistically significant effect in relationship to treatments, $F(7, 127) = 2.73$, $p < 0.05$, but there were no significant differences between intensities, $F(7, 15) = 0.83$, NS. Dunnett's *t*-tests showed statistically nonsignificant effects with most of the doses, which increased the number of electric shocks received

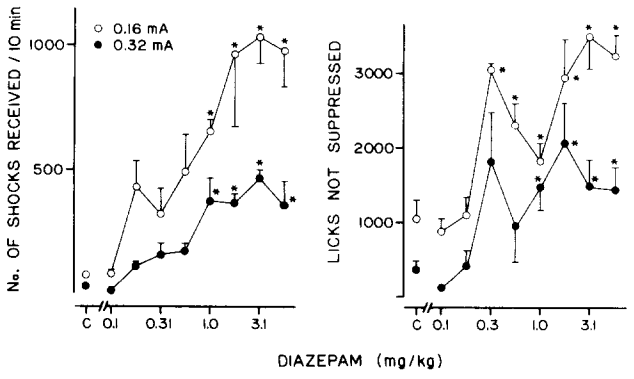

FIG. 1. Effects of diazepam on the conflict test. Diazepam was injected (IP) 30 min before conflict test. Shown are the mean ± SEM of number of electric shocks received (left panel) and the licks not suppressed (right panel). Electric shock intensity of 0.16 mA (○) and 0.32 mA (●). *$p < 0.05$ vs. vehicle-injected controls. $n = 8$ animals per group.

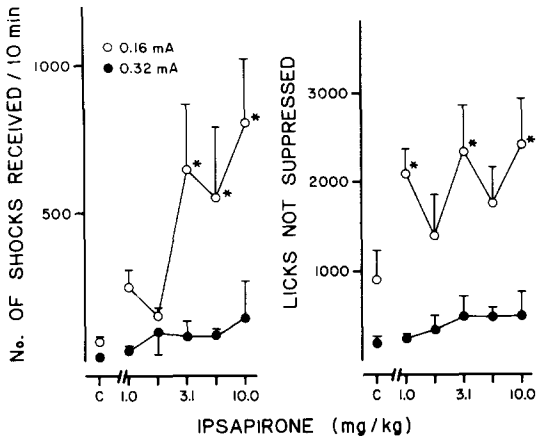

FIG. 2. Effects of ipsapirone on conflict test. Ipsapirone was administered (IP) 30 min before conflict test. n = 8-16 animals per group. See details as in Fig. 1.

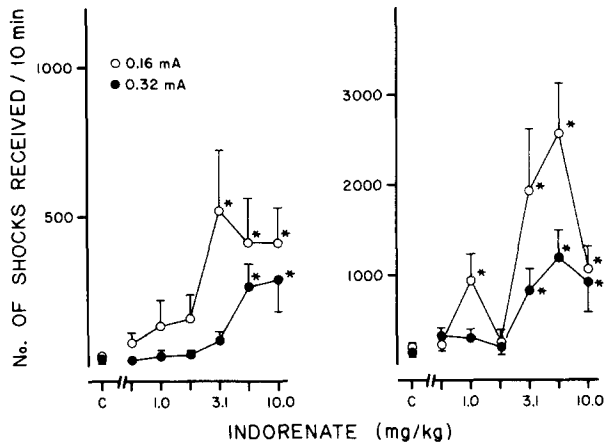

FIG. 4. Effects of indorenate on conflict test. Indorenate was administered (IP) 90 min before conflict test. n = 8 animals per group. See details as in Fig. 1.

at the intensity of 0.16 mA and at the intensity of 0.32 mA except for the dose of 10.0 mg/kg. The number of licks not suppressed were increased at both intensities.

The ANOVA for indorenate (Fig. 4) revealed statistically significant effects between the intensities, $F(1, 112) = 12.13$, $p < 0.01$, and doses $F(1, 112) = 12.13$, $p < 0.01$. Dunnett's t-tests of indorenate show statistically significant increments in the number of electric shocks received at doses from 3.1 to 10.0 mg/kg at 0.16 mA, and at doses from 5.6 to 10.0 mg/kg at 0.32 mA. A similar finding was observed with the licks not suppressed.

The main findings of these experiments are the doses with a statistically significant anticonflict effect and the doses producing maximal effects for each of the four drugs. In this sense, the order of anticonflct effects observed in the intensity of 0.16 mA were: diazepam > buspirone > ipsapirone > indorenate; and for the intensity of 0.32 mA were: diazepam > indorenate > buspirone.

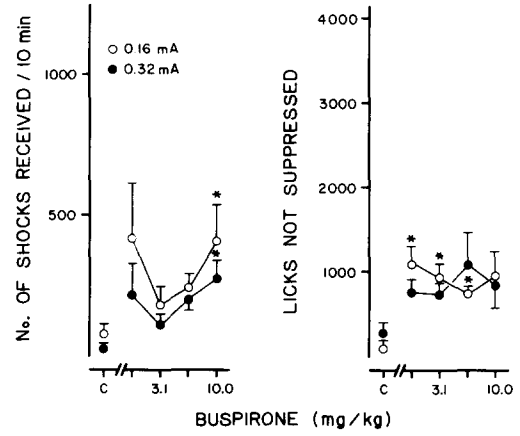

FIG. 3. Effects of buspirone on conflict test. Buspirone was administered (IP) 30 min before conflict test. n = 8-16 animals per group. See details as in Fig. 1.

DISCUSSION

The results with diazepam confirm the sensitivity and reliability of the conflict test version used in the present study. The antianxiety effects obtained with diazepam in a very wide range of doses with both electric shock intensities (0.16 and 0.32 mA) have clarified this point. The anticonflict effects of the 5-HT$_{1A}$ agonists were weaker than diazepam. On the other hand, the number of licks not suppressed by diazepam and indorenate were increased in a dose-dependent manner, while they was increased irregularly by ipsapirone and buspirone with the two shock intensities. These results do not rule out the possibility that the drugs employed here increased water intake in addition to their ability to increase the number of electric shocks received, since evidence exists that some 5-HT$_{1A}$ agonists (8-OH DPAT and gepirone) increase postinjection water intake (14). However, in the cases of buspirone and ipsapirone, such effect did not reach statistically significant levels (14) nor increase water intake in normal, deprived, or presatiated animals (3,17,21). Therefore, licks not suppressed can be independently affected in conflict tests, at least during conflict acquisition.

There are contradictory findings related to the anxiolytic effects of 5-HT$_{1A}$ agonists in several animal models of anxiety (9,12). This seems to be particulary true in the case of buspirone (2,10,16-18,22,24,26,28,30) and ipsapirone (4,7,17,18, 35). Such discrepancies may be due to methodological differences used by each author.

In the conflict test, the procedures usually employ the delivery of chronic electric shocks to controll or eliminate the engendered effects by the compounds that increase intake. However, it has been found that compounds decreasing 5-HT transmission, such as 5,7-DHT, pCPA, methysergide, or ciproheptadine, are inactive in a chronic conflict test but they are active during conflict acquisition or an acute conflict [see (33) for review].

Experiments carried out with 5-HT$_{1A}$ agonists in conflict test, using either chronic or acute procedures, have been employed with either adjusted or fixed electric shock intensities. It is important to emphasize that from experiments in which chronic and adjusted electric shock intensities were used (2,7,16,28), only one author reported anticonflict effects with

buspirone (28); on the other hand, from those experiments employing acute administration and fixed shock intensities, only one (31) was unable to find anticonflict effects with buspirone, while others found anticonflict effects with buspirone or ipsapirone (4,10,18,23-26), but always to a lesser degree than with benzodiazepines. It is important to point out that when 8-OH-DPAT was tested using a fixed intensity of 0.16 mA for supression of lick responses, an effective and dose-related anticonflict effect was found (11), as observed in the present experiments.

It has been reported that rats trained with gradual increments in electric shock intensity showed less anxiolytic effects than those trained with a fixed intensity (1). The present results show that three 5-HT_{1A} agonists were active with a low shock intensity, while at the higher intensity, their anticonflict effects were of lesser degree or were absent (ipsapirone). It was recently reported (19) that buspirone showed inconsistent anticonflict effects in the Geller-Seifter conflict test when increasing shock intensities were used in a wide range of experimental conditions. The individual adjustment of the electric shock intensity allows the establishment of similar suppression levels in the response output, but this by no means attains the same level of anxiety between animals. The present results constitute an evaluation of this phenomenon, since in rats trained with the electric shock intensity of 0.16 mA, the four drugs were active; with the higher intensity (0.32 mA), the anticonflict effects were smaller or absent. These results suggest that the electric shock adjustment in the conflict test is difficult for the proper pharmacological evaluation of 5-HT_{1A} agonists with anxiolytic properties.

The antianxiety effects of 5-HT_{1A} agonists in other animal models of anxiety are conflicting (5,9,19,34). However, there is at least one paper reporting that 5-HT_{1A} agonists are active on social interaction test, potentiated startle response, two-compartment test, and passive avoidance response, and these agonists have no effect on elevated plus-maze, shock probe, and conditional emotional response (5,9,19,34).

Several factors can be related to inconsistencies, since 5-HT_{1A} agonists alter diverse behavioral aspects [see (22) for review], such as consummatory respones, activity, pain perception, etc. Besides, it is noteworthy that a given behavioral test can activate specific 5-HT pathways. Therefore, it is necessary to take into account if the drug is acting at a pre- or postsynaptic level, as agonist or antagonist, and its possible interactions with diverse neurotransmission systems. On the other hand, it is necessary to take into account what kind of behavioral responses an animal needs to deal with a specific anxiety test, and what structures and neurotransmission responses are active in the behavioral test per se.

ACKNOWLEDGEMENTS

This work was partially presented at the 21st Annual Meeting of Society for Neuroscience, November 10–15, 1991, New Orleans, LA.

REFERENCES

1. Anderson, D. C.; Crowell, C. R.; Ramirez, R. Stepwise reduction in US intensity and density: Maintenance and extinction of conditioned supression. Anim. Learn. Behav. 15:312–320; 1987.
2. Budhram, P.; Deacon, R.; Gardner, C. R. Some putative non-sedating anxiolytics in a conditioned licking conflict. Br. J. Pharmacol. 88:331P; 1986.
3. Cooper, S. J.; Fryer, M. J.; Neill, J. C. Specific effect of putative 5-HT_{1A} agonists, 8-OH-DPAT and gepirone, to increase hypertonic saline consumption in the rat: Evidence against a general hyperdipsic action. Physiol. Behav. 43:533–537; 1988.
4. Chojnacka-Wojcik, E.; Przegalinski, E. Evidence for the involvement of 5-HT_{1A} receptors in the anticonflict effect ofipsapirone in rats. Neuropharmacology 30:703–709; 1991.
5. Chopin, P.; Briley, M. Animal models of anxiety: The effect of compounds that modify 5-HT neurotransmission. Trends Pharmacol. Sci. 8:383–388; 1987.
6. Dantzer, R. Behavioral analysis of anxiolytic drug action. In: Greenshaw, A. J.; Dourish, C. T., eds. Experimental psychopharmacology. New York: Human Press; 1987:263–297.
7. Deacon, R.; Gardner, C. R. Benzodiazepine and 5-HT ligands ina rat conflict test. Br. J. Pharmacol. 88:330P; 1986.
8. Dompert, W. V.; Glaser, T.; Traber, J. H-TVXQ 7821: Identification of 5-HT_{1A} binding sites as target for a novel putative anxiolytic. Naunyn Schmiedebergs Arch. Pharmacol. 328:467–470; 1985.
9. Dourish, C. T. Brain 5-HT_{1A} receptor and anxiety. In: Dourish, C. T.; Ahlenius, A.; Hutson, P. H., eds. Brain 5-HT_{1A} receptors. Chischester, UK: Ellis Horwood; 1987:261–278.
10. Eison, A. S.; Eison, M. S.; Stanley, M.; Riblet, L. A. Serotonergic mechanisms in the behavioral effects of buspirone and gepirone. Pharmacol. Biochem. Behav. 24:701–707; 1986.
11. Engel, J. A.; Hjorth, S.; Svensson, K.; Carlsson, A.; Liljequist, S. Anticonflict effect of the putative serotonin receptor agonists 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). Eur. J. Pharmacol. 105:365–368; 1984.
12. File, S. E. Beyond the benzodiazepines: The search for new anxiolytics. Hum. Psychopharmacol. 2:151–158; 1987.
13. Geller, I.; Seifter, J. The effects of mebrobamate, barbitures, *d*-amphetamine and promazine on experimentally induced conflict in the rat. Psychopharmacologia 1:482–492; 1960.
14. Gilbert, F.; Dourish, C. T. Effects of the novel anxiolytics gepirone, buspirone and ipsapirone on free feeding and on feeding induced by 8-OH-DPAT. Psychopharmacology (Berlin) 93:349–352; 1987.
15. Glesson, S.; Ahlers, S. T.; Mansbach, R. S.; Foust, J. M.; Barrett, J. E. Behavioral studies with anxiolytic drugs. VI. Effects on punished responding of drugs interacting with serotonin receptor subtypes. J. Pharmacol. Exp. Ther. 250:809–817; 1989.
16. Goldberg, M. E.; Salama, A. I.; Patel, J. B.; Malick, J. B. Novel non-benzodiazepine anxiolytics. Neuropharmacology 22:1499–1504; 1983.
17. Higgins, G. A.; Bradbury, A. J.; Jones, B. J.; Oakley, N. R. Behavioral and biochemical consequences following activation of 5-HT_{1A}-like and GABA receptors in the dorsal raphé nuclues of the rat. Neuropharmacology 27:993–1001; 1988.
18. Higgins, G. A.; Jones, B. J.; Oakley, N. R. Effects of 5-HT_{1A} receptor agonists in two models of anxiety after dorsal raphe injection. Psychopharmacology (Berlin) 106:261–267; 1992.
19. Howard, J. L.; Pollard, G. I. Effects of buspirone in the Geller-Seifter conflict test with incremental shock. Drug Dev. Res. 19: 37–49; 1990.
20. Hoyer, D.; Engel, G.; Kalkman, H. O. Molecular pharmacology of 5-HT_{1A} and 5-HT_2 recognition sites in rat and pig brain membranes: Radioligand binding studies with [^{3}H] 5-HT, [^{3}H] 8-OH-DPAT, (−)[^{125}I] iodocyanopindolol, [^{3}H] mesulergine and [^{3}H] ketanserin. Eur. J. Pharmacol. 118:13–23; 1985.
21. Lucki, I. Rapid discrimination of the stimulus properties of 5-hydroxytryptamine agonists using conditioned taste aversion. J. Pharmacol. Exp. Ther. 247:1120–1127; 1988.
22. Lucki, I.; Wieland, S. 5-Hydroxytryptamine$_{1A}$ receptors and behavioral responses. Neuropsychopharmacology 3:481–493; 1990.
23. Mason, P.; Skinner, J.; Luttinger, D. Two tests in rats for antianxiety effect of clinically anxiety attenuating antidepressant. Psychopharmacology (Berlin) 92:30–34; 1987.

24. McCloskey, T. C.; Paul, B. K.; Commissaris, R. L. Buspirone effects in an animal conflict procedure: Comparison with diazepam and phenobarbital. Pharmacol. Biochem. Behav. 27:171–175; 1987.
25. Moser, P. C.; Tricklebank, D. N.; Middlemiss, A. K.; Mir, M. F.; Fozard, J. R. Characterization of MDL 73005EF as a 5-HT_{1A} selective ligand and its effects in animal models of anxiety: Comparision with buspirone, 8-OH-DPAT and diazepam. Br. J. Pharmacol. 99:343–349; 1990.
26. Oakley, N. R.; Jones, B. J. Buspirone enhances flunitrazepam binding in vivo. Eur. J. Pharmacol. 87:499–500; 1983.
27. Peroutka, S. J. The molecular pharmacology of 5-hydroxytryptamine receptor subtypes. In: Peroutka, S. J., ed. Serotonin receptor subtypes. New York: Wiley-Liss; 1991:65–80.
28. Pich, E. M.; Samanin, R. Disinhibition effects of buspirone and low doses of sulpiride and haloperidol in two experimental anxiety models in rats: Possible role of dopamine. Psychopharmacology (Berlin) 89:125–130; 1986.
29. Pollard, G. T.; Howard, J. L. Effects of drugs on punished behavior: Pre-clinical test for anxiolytics. Pharmacol. Ther. 45:403–424; 1990.
30. Pollard, G. T.; Howard, J. The Geller-Seifter conflict paradigm with incremental shock. Psychopharmacology (Berlin) 62:117–121; 1979.
31. Sanger, D. J.; Joly, D.; Zikovic, B. Behavioral effects of nonbenzodiazepine anxiolytic drugs: A comparasion of CGS 9896 and zaplicone with chlordiazepoxide. J. Pharmacol. Exp. Ther. 232: 831–837; 1985.
32. Schefke, D. M.; Fontana, D. J.; Commissaris, R. L. Anti-conflict efficacy of buspirone following acute versus chronic treament. Psychopharmacology (Berlin) 99:427–429; 1989.
33. Soubrie, P. Reconciling the role of central serotonin neurons in humans and animal behavior. Behav. Brain Sci. 9:319–364; 1986.
34. Traber, J.; Glaser, T. 5-HT_{1A} receptor-related anxiolytics. Trends Pharmacol. Sci. 8:432–437; 1987.
35. Traber, J.; Schuurman, T.; Benz, U. A comparision of the 5-HT_{1A} receptor related anxiolytics buspirone, gepirone, ipsapirone, SM-3997, their common metabolite 1-PP and diazepam in animals models of anxiety. Psychopharmacology (Berlin) 96(Suppl.) 352; 1988.
36. Treit, D. Animals models for the study of anti-anxiety agents: A review. Neurosci. Biobehav. Rev. 9:203–222; 1985.
37. Vogel, J. R.; Beer, B.; Clody, D. E. A simple and reliable conflict procedure for testing antianxiety agents. Psychopharmacologia 21:8–12; 1971.