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Effects of Buspirone, Diazepam, and Zolpidem on Open Field Behavior, and Brain [^{3}H]Muscimol Binding After Buspirone Pretreatment

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SIEMIĄTKOWSKI, M., H. SIENKIEWICZ-JAROSZ, A. I. CZŁONKOWSKA, A. BIDZIŃSKI AND A. PŁAŹNIK. *Effects of buspirone, diazepam, and zolpidem on open field behavior, and brain [^{3}H]muscimol binding after buspirone pretreatment.* PHARMACOL BIOCHEM BEHAV **66**(3) 645–651, 2000.—The effects of 5-HT_{1A} receptor agonist buspirone, a nonselective (diazepam), and a selective (zolpidem) $GABA_A$ receptor agonist were compared in the open field test of neophobia. Unhabituated rats were pretreated with the drugs once, prior to a first exposure to the open field, and their behavior was recorded both during this test and during a second trial 24 h later. It has been hypothesized that the decrease in exploratory activity observed during the second test session may be considered an adaptive reaction to the first day aversive experience (neophobia). If so, a selective modulation of 5-HT and GABA systems activity during the test could bring about significant changes in animal behavior on the retest. Buspirone at the lowest dose of 0.3 mg/kg revealed anxiolytic-like properties on the first day, whereas the action of diazepam and zolpidem was modulated by the dose-related sedative effect. At the dose of 2.4 mg/kg buspirone elicited delayed in time anxiolytic-like action, i.e., produced the antithigmotactic effect during the retrial 24 h later. Diazepam and zolpidem failed to exhibit similar profile of action. Autoradiography of [^{3}H]muscimol binding after pretreatment of rats with buspirone showed a significant increase in the selective radioligand binding within the frontal cortex and a similar, near-significant tendency in the dentate gyrus of the hippocampus. The behavioral data validate buspirone as important drug for the treatment of anxiety disorders, devoid of disruptive influence on motor and cognitive processes. The open field test, as modified by us, appeared sensitive in distinguishing the behavioral profiles of action of different anxiolytic compounds, including 5-HT_{1A} receptor agonist. The present results support the assumption that reduced turnover of 5-HT due to stimulation of 5-HT_{1A} autoreceptors, may bring about changes in $GABA_A$ receptor system activity, in some brain structures, leading to the anxiolytic effect. © 2000 Elsevier Science Inc.

FULL, nonselective agonists at $GABA_A$ receptor complex are of great relevance for clinical treatment of anxiety states; however, their adverse effects stimulate the search for new drugs with better benefit/risk ratio. On the one hand, the search is focused on assessment of selective $GABA_A$ receptor subtypes agonists, e.g., zolpidem and quazepam. Preclinical data suggest their lower tolerance and dependence liability, and they indicate a lower propensity to induce serious central adverse effects (29,30). However, compounds with a higher selective affinity for the type 1 of benzodiazepine binding site ($GABA_{A1a}$) are indicated mainly for the treatment of insomnia. On the other hand, the search is directed towards nonbenzodiazepine compounds, with different neurochemical profiles, only indirectly influencing GABA system activity. In

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this context, drugs which directly or indirectly diminish 5-HT neurotransmission seem to be of special interest given that there is a growing body of evidence indicating functional interaction between brain 5-HT and GABA systems in the control of emotional processes (3,7,23,37,38,39). Decreased 5-HT, and increased GABA neurotransmission produce anxiolytic-like effects in different animal models of anxiety. Furthermore, $GABA_A$ receptor was suggested, in the literature data, as a possible site of 5-HT/GABA systems interaction, particularly when it was revealed that $GABA_A$ receptor complex antagonists, bicuculline, picrotoxin, and flumazenil counteracted the antianxiety action of 5-HT depletion (20,38). The $5\text{-}HT_{1A}$ autoreceptor agonists buspirone and flesinoxan are the best known nonbenzodiazepine compounds with anxiolytic potential, as revealed in clinical practice. Unfortunately, these clinically effective drugs yielded disparate results in several conventional animal screening procedures (elevated plus-maze, the Vogel test, aversive brain stimulation), indicating the need for other tests, with better predictive potential (15,32,42). Moreover, the mechanism of anxiolytic action of buspirone and flesinoxan remains unclear.

Taking it all into consideration, the aim of the present study was to examine the effects of nonselective $GABA_A$ receptor agonist-diazepam, $GABA_{A1a}$-selective agonist zolpidem, and $5\text{-}HT_{1A}$ receptor agonist buspirone, on anxiety-related behavior, and to investigate the possible 5-HT/GABA interactions underlying such effects. In the present study we used the rat neophobic reaction to a novel environment in the open field test (OFT), modified by including an additional test session. Drugs were administered prior to a first exposure and behavior was recorded both during this test and during a second (drug-free) trial 24 h later. It has been hypothesized that the decrease in exploratory activity on the second test session may be considered an adaptive reaction to the first day aversive experience (neophobia). If so, a selective modulation of activity of 5-HT and GABA systems during the test could bring about significant changes in animal behavior on the retest. The obtained data were analyzed in terms of thigmotaxis, i.e., the tendency to stay close to walls of a novel environment (36,43,45), and changes ensuing after the administration of buspirone in the binding of [^{3}H]muscimol —a $GABA_A$ receptor agonist—to some brain structures, were measured with the help of quantitative autoradiography.

METHOD

Animals

Male Wistar rats (200 ± 20 g), bought from a licensed breeder, were housed in standard laboratory conditions under a 12 h light/dark cycle (lights on at0600 h), in a constant temperature (21 ± 2°C) and 70% humidity. The animals were kept in pairs in cages (60/30/20 cm) with free access to food and tap water. All experimental procedures using animal subjects were approved by the Committee for Animal Care and Use at the Institute of Psychiatry and Neurology in Warsaw.

Drugs and Treatment

Diazepam (Polfa, Poland), zolpidem (Synthelabo, France), and buspirone (Astra, Sweden) were administered intraperitoneally (IP) (2 ml/kg) suspended in 1% Tween (diazepam, zolpidem) or dissolved in 0.9% NaCl (buspirone) 30 min before the first testing day in the OFT. In another experiment, some behaviorally active doses of the drugs, established in the experiment with pretest drug injections, were administered immediately after the test. The behavior of all animals was evaluated during the retest 24 h later. Control rats received an appropriate volume of respective vehicle. Each group contained 9–10 rats. The experiments were conducted sequentially with only one of the treatments at any given time. Autoradiography of [^{3}H]muscimol binding was preceded with administration of buspirone at the dose of 2.4 mg/kg IP 30 min prior to decapitation.

Open Field Test

The open field apparatuses used in this experiment consisted of two round arenas (80-cm diameter) with 30-cm high walls, each equipped symmetrically with three photocells mounted 80 cm apart and 4 cm above the floor level. The test was performed in a soundproof chamber under dim light and continuous white noise (65 dB) without previous habituation. General activity (number of photobeam interruptions) was scored automatically by a cumulative recorder for 15 min. The number of central entries (defined as a movement of an animal from the wall to the central area over a distance of approximately 14 cm) was scored by an experimenter via closed-circuit television. The antithigmotactic effect was calculated as a ratio of the number of entries into central part to the rat locomotor activity and multiplied by 1000. The higher value of the score the lower thigmotaxis and the more pronounced anxiolytic-like effect. This parameter was calculated for each rat separately, then the mean value for each experimental group was received. The open field sessions were performed for 2 consecutive days.

Autoradiography

After the animals were sacrificed, their brains were rapidly removed and frozen in isopentane (−30 to −40°C) cooled with dry ice. A detailed description of the method for receptor autoradiography has been published (3,19). Briefly, the whole brains were stored at −70°C. Coronal sections (12 μm) were cut on a cryostat at −20°C according to the atlas of the rat brain (28), and thaw-mounted onto gelatin-coated glass slides. Sections were stored at −20°C until assay (1 to 2 days). Slides were preincubated in 50 mM Tris-citrate buffer (pH 7.1) for 20 min at 4°C to remove endogenous competitors. After being dried, they were incubated in the same buffer as used for the preincubation but supplemented with 5 nM [^{3}H]muscimol (19.1 Ci/mmol, Amersham) for 40 min at 4°C. Nonspecific binding was determined in the presence of 0.2 mM GABA. The sections were then washed in the cold buffer for 1 min and quickly in-out dipped in distilled water. The slides were dried under a cold stream of air, placed in X-ray cassettes, and exposed to tritium-sensitive film ([^{3}H]Hyperfilm, Amersham) at 4°C together with standards ([^{3}H]microscale, Amersham). After exposure for 6 weeks the films were developed with a Kodak LX-24 developer for 57–7 min, fixed, washed and dried. The autoradiogram was placed in a white light transilluminator (Sigma, St. Louis, MO, USA) to measure the densitometrically determined optical density values. Quantitative analysis of the autoradiogram was performed with an image analysis system (Analytical Imaging Station, Imaging Research Inc., St. Catharines, Canada). For each film, the best fit of the film densities produced by radioactive Amersham standards to a 4th degree polynomial was generated by the computer as a standard curve. Subsequently, this standard curve was used to convert optical densities produced by selected brain regions into amount of radioligand bound

(nCi/mg tissue). [^{3}H]Muscimol nonspecific binding was negligible. Each group contained 9 to 10 animals.

Statistical Analysis

With the exception of autoradiographical scores analyzed by Student's *t*-test for independent samples, data were submitted to two-factor analysis of variance (ANOVA) (independent treatment factor, repeated measures for days factor), followed by Newman-Keuls post hoc test. The confidence limit of $p < 0.05$ was considered statistically significant.

RESULTS

Diazepam

Two-way analysis of variance showed that there was a significant main effect of days on the rats' motor behavior [$F(1, 44) = 33.95$, $p < 0.001$], and the number of central entries [$F(1, 43) = 7.29$, $p < 0.01$]. Post hoc analysis revealed the inhibitory influence of diazepam administered acutely at the dose of 0.05 mg/kg ($p < 0.01$), 0.2 mg/kg ($p < 0.05$), and 1.5 mg/kg ($p < 0.01$) on rats ambulation (Table 1). Analysis of day-to-day changes indicated that there was a significant suppression of motor activity across two testing days only in the group of control animals ($p < 0.01$) (Table 1, Fig. 1). Two-way analysis of variance did not show any other significant main effect of the drug, days nor diazepam x days interaction on thigmotaxis, and the number of central entries into the central sector of the open field (Table 1, Fig. 2).

Zolpidem

Two-way analysis of variance showed a significant main effect of zolpidem on the rats' motor activity [$F(3, 36) = 7.74$, $p < 0.001$], thigmotaxis [$F(3, 33) = 3.43$, $p < 0.05$], and the number of central entries [$F(3,35) = 4.13$, $p < 0.05$] (Table 1). Two-way ANOVA indicated also a significant main effect of days on the number of central entries [$F(1, 35) = 6.80$, $p < 0.05$], and a significant drug x day interaction in modulating rats locomotor activity [$F(3, 36) = 11.33$, $p < 0.001$], and the number of central entries [$F(3, 35) = 9.43$, $p < 0.001$] (Table 1, Fig. 1). Post hoc analysis of the data revealed that zolpidem (2.0 mg/kg) significantly decreased rats' motor activity on the first testing day ($p < 0.01$) (Table 1). Within group comparisons showed that a significant decrease in rat motility on the second day occurred only in the control ($p < 0.05$) and zolpidem (0.1 mg/kg, $p < 0.001$) pretreated animals (Table 1, Fig. 1). On the other hand, zolpidem given at the dose of 2.0 mg/kg significantly stimulated rat locomotion on the following day ($p < 0.01$) (Table 1, Fig. 1). The number of central entries was increased after the dose 0.1 mg/kg ($p < 0.001$) and decreased after the dose of 2.0 mg/kg ($p < 0.05$) of zolpidem, when the effects were examined immediately after drug injection (Table 1). A significant increase in the number of central entries was evident in the between-group comparisons on the test ($p < 0.001$) and retest ($p < 0.05$), after administration of zolpidem at the dose of 0.1 mg/kg (Table 1). Post hoc test did not show any significant changes in thigmotactic behavior of rats (Table 1, Fig. 2).

Buspirone

Two-way analysis of variance showed that there was a significant main effect of days [$F(1, 35) = 18.03$, $p < 0.001$], and buspirone x days interaction [$F(3, 35) = 5.84$, $p < 0.01$] on the rats' motor behavior in the open field test (Table 1). In comparison with the control group, buspirone dose-dependently disinhibited animals' motility 24 h after drug injection, with the dose of 2.4 mg/kg producing the most potent effect ($p < 0.001$) (Table 1, Fig. 1). Two-way analysis of variance indicated an overall significant effect of the drug and drug x day interaction on thigmotaxis [drug: $F(3, 33) = 3.29$, $p < 0.05$; drug x days interaction: $F(3, 33) = 4.08$, $p < 0.05$], and the number of central entries [drug: $F(3, 35) = 2.95$, $p < 0.05$; drug x days interaction: $F(3, 35) = 9.34$, $p < 0.001$] (Table 1, Fig. 2). Post hoc analysis of data revealed an enhancement of the antithigmotactic effect on the second day, after the doses of 1.2 mg/kg ($p < 0.05$) and 2.4 mg/kg ($p < 0.01$) (Table 1, Fig. 2). The number of central entries was increased immediately after injection of 0.3 mg/kg of buspirone ($p < 0.05$) and on the

TABLE 1

THE EFFECTS OF DIAZEPAM, ZOLPIDEM, AND BUSPIRONE IN THE OPEN FIELD TEST

Group	Motor Activity Day 1	Motor Activity Day 2	Entries Into Central Sector—Day 1	Entries Into Central Sector—Day 2	Anti-Thigmotactic Effect—Day 1	Anti-Thigmotactic Effect—Day 2
Control (1% Tween)	112.9 ± 20.1	74.2 ± 12.6##	4.6 ± 1.5	2.9 ± 1.4	35.3 ± 8.3	20.0 ± 8.9
Diazepam 0.05 mg	82.2 ± 10.8**	54.9 ± 12.4	1.4 ± 0.4	3.4 ± 1.5	21.2 ± 7.5	40.2 ± 17.1
Diazepam 0.2 mg	81.0 ± 9.4*	54.1 ± 13.8	4.1 ± 1.0	2.6 ± 1.2	48.6 ± 8.4	28.8 ± 10.5
Diazepam 0.8 mg	93.7 ± 16.1	76.4 ± 15.2	6.7 ± 1.8	2.9 ± 0.8	64.6 ± 8.8	36.3 ± 11.2
Diazepam 1.5 mg	63.3 ± 9.6**	47.6 ± 6.5	4.3 ± 1.3	2.0 ± 0.6	41.5 ± 11.2	31.9 ± 11.9
Control (1% Tween)	82.9 ± 5.1	59.5 ± 7.7#	5.0 ± 1.2	1.2 ± 0.4#	58.4 ± 39.3	38.5 ± 14.7
Zolpidem 0.1 mg	99.8 ± 5.8	59.1 ± 7.0##	8.5 ± 2.1**	4.3 ± 1.7##,*	84.5 ± 18.3	68.2 ± 22.4
Zolpidem 1.0 mg	65.8 ± 5.2	70.2 ± 12.0	2.6 ± 0.7	3.2 ± 0.5	30.5 ± 8.0	47.7 ± 10.1
Zolpidem 2.0 mg	30.1 ± 6.2**	54.9 ± 5.9##	0.9 ± 0.6*	2.6 ± 0.8	8.9 ± 6.7	40.1 ± 11.7
Control (saline)	65.7 ± 5.5	32.6 ± 6.3##	5.5 ± 1.2	3.0 ± 1.0	77.4 ± 12.3	66.0 ± 18.3
Buspirone 0.3 mg	79.8 ± 7.8	53.4 ± 7.9#,**	10.9 ± 0.9*	5.4 ± 1.3#	113.8 ± 9.4	96.6 ± 18.3
Buspirone 1.2 mg	71.8 ± 5.8	57.9 ± 6.0**	5.6 ± 1.1	7.7 ± 1.4*	79.6 ± 45.5	125.3 ± 13.5*
Buspirone 2.4 mg	64.2 ± 6.9	71.1 ± 4.7**	6.6 ± 1.3	11.8 ± 2.8#,**	100.7 ± 13.1	148.7 ± 24.7**

Motor activity, the number of central entries and anti-thigmotactic effect (calculated as a ratio of the number of entries into the central part of the open field to the rat locomotor activity, and multiplied by 1000). The anti-thigmotactic ratio was calculated for each rat separately and then the mean value for each experimental group was received. The drugs were administered IP 30 min before the first testing day. The number of rats in each experimental group varied from 9 to 10. The data are shown as means ± SEM *-differs from respective control, "#" = day-to-day, within group comparisons. *, # = $p < 0.05$; **,## = $p < 0.01$.

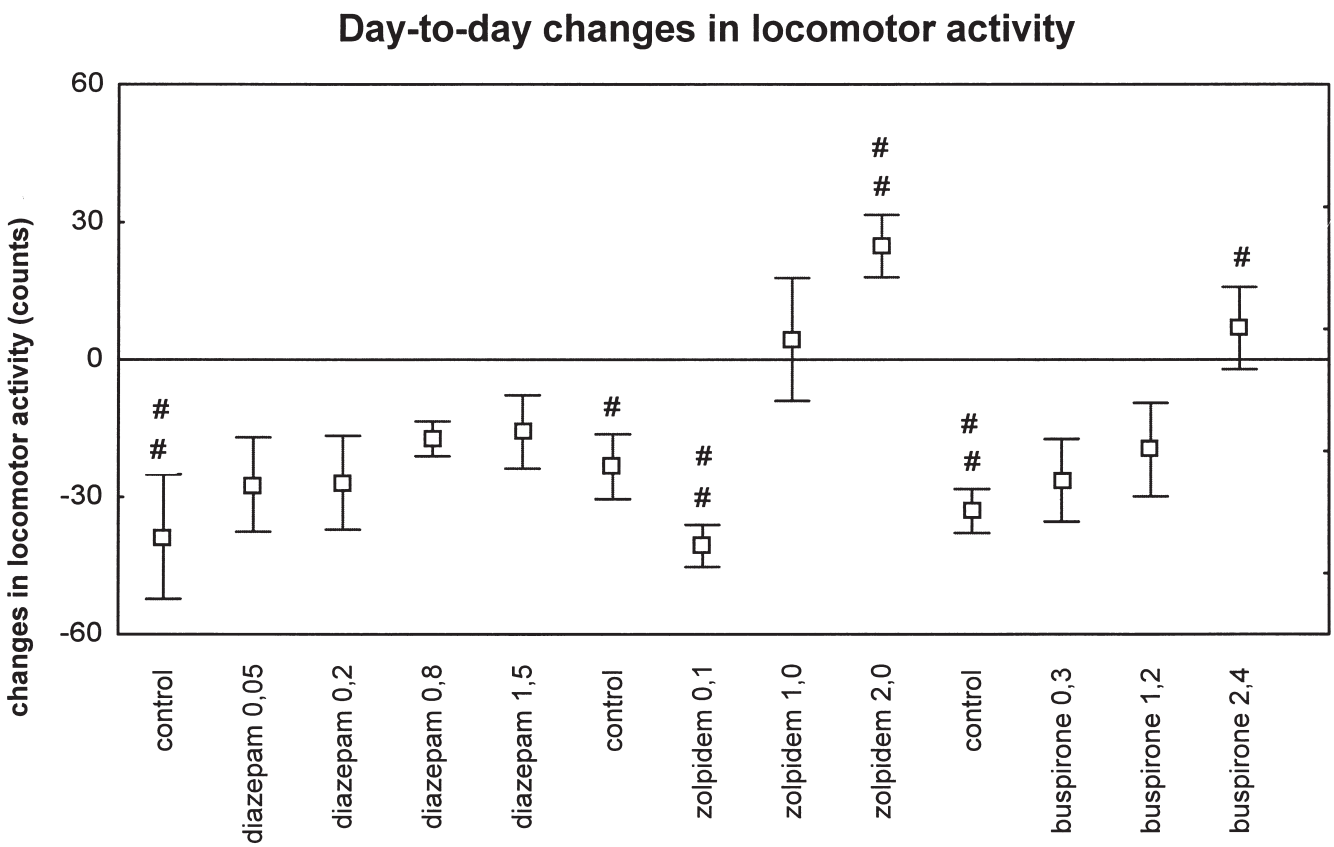

FIG. 1. Day-to-day changes in ambulatory activity of rats subjected to two daily sessions in the open field test. The drugs were administered once, 30 min before the first session. The number of rats in each experimental group varied from 9 to 10. The data are shown as means ± SEM "#" = day-to-day, within group comparisons. # = $p < 0.05$; # # = $p < 0.01$.

following day after the dose of 1.2 mg/kg ($p < 0.005$) and 2.4 mg/kg ($p < 0.0001$) of the drug (Table 1). Within group comparison revealed a significant increase in the number of central entries on the second day following administration of buspirone at the dose of 2.4 mg/kg ($p < 0.05$) (Table 1).

Post-test injections of the drugs revealed no alterations, in comparison to appropriate control groups of animals, in activity and exploratory scores during the test (Table 2). Post hoc analysis of data showed only a significant decrease in exploratory activity on the second day (retest) in all groups of examined animals (Table 2).

Autoradiography of [^{3}H]muscimol binding showed a statistically significant, higher specific binding of radioligand to the $GABA_A$ receptor in the frontal cortex only [by 14%, $t = 2.14$, $p < 0.05$] and a near-significant increase in binding within the dentate gyrus of the hippocampus [by 14%, $t = 1.89$, $p = 0.08$] (Table 3). No differences in the specific binding of [^{3}H]muscimol were found in other brain structures examined (nucleus accumbens, caudate putamen, dorsal raphe nucleus).

DISCUSSION

The significant decrease in exploratory activity of the control animals on the second test session can be considered an adaptive reaction to the first day aversive experience. It can be reasoned that anxiolytic drugs would make the first day session less stressing, thus affecting changes in day-to-day motility and exploration. If so, the anxiolytic-like effect might be demonstrated when recording the open field parameters 24 h following the treatment. Importantly, the lack of significant alterations in rats behavior after post-test injections of the drugs puts into question the role that disturbances in learning and memory might play in the day-to-day changes following administration of $GABA_A$ receptor ligands and buspirone. Furthermore, the effect of buspirone, emerging on the second test session, can not be interpreted in terms of amnesia, as diazepam did not reveal similar changes. The most clear anti-emotional effect was observed after administration of buspirone. The drug at the dose of 1.2 and 2.4 mg/kg blocked the day-to-day behavioral changes as well as significantly increased rats ambulation and the number of central entries on the retest, in comparison to untreated animals. Diazepam at different doses and zolpidem at the dose of 1.0 mg/kg also antagonized day-to-day decrease in rats motility observed in naive animals (Fig. 1). This can be considered an anxiolytic-like effect related to the reduction of the first day aversive experience (neophobia) by the pretest administered drugs. The action of zolpidem at the dose of 2.0 mg/kg was affected by its marked first-day sedative effect. Thus the applied method appeared sensitive enough to differentiate the anxiolytic vs. sedative profiles of action of various doses of psychotropic agents. Given the results above, buspirone revealed the most clear anxiolytic-like effect on the second day, whereas the action and of both benzodiazepine-receptor ligands was less selective, and particularly at higher doses, affected by the sedative effect on the first day test. Buspirone was the only drug that at the nonsedative doses inhibited or even inverted the day-to-day decrement in motility and exploratory scores. In turn,

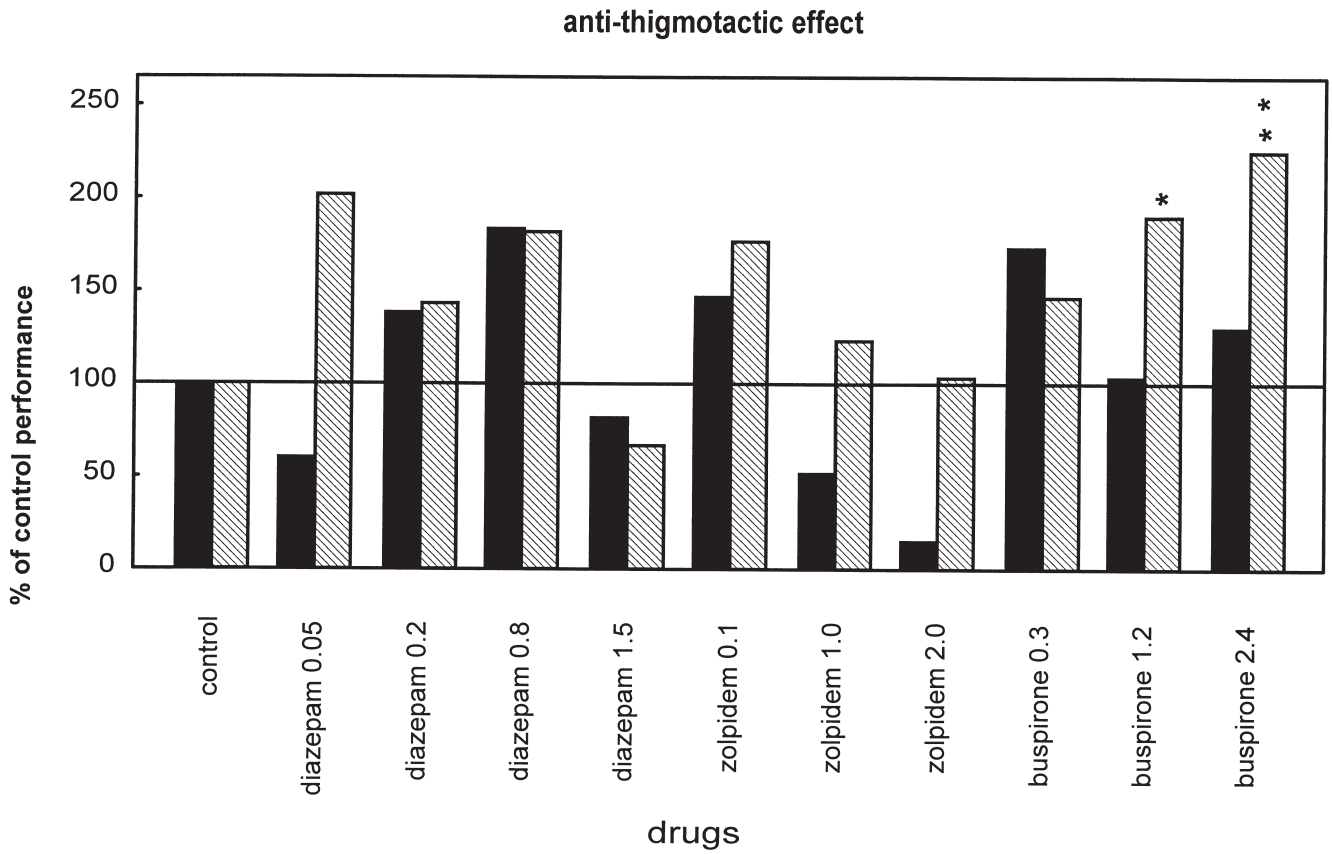

FIG. 2. The anti-thigmotactic effect during the test (black bars) and retest (hatched bars) calculated as a ratio of the number of entries into the central arena of the open field to the rat locomotor activity, and multiplied by 1000 (the higher value - the lower thigmotaxis, and the more pronounced anxiolytic-like effect). The anti-thigmotactic ratio was calculated for each rat separately and then the mean value for each experimental group was received. The results are given as % of performance of respective control group. The number of rats in each experimental group varied from 9 to 10. * - differs from control. * = $p < 0.05$, ** = $p < 0.01$, and it refers to absolute values (not a percentage presentation) of thigmotaxis.

diazepam (except the dose of 0.8 mg/kg) and zolpidem at the dose of 2.0 mg/kg had inhibited rat motor activity on the first day, thus indicating their contribution to unspecific (antiarousal), apart from specific (antiemotional), processes. Another point requiring a comment is the variability of the control groups results. This is most probably due to the fact that different solvents were used (1% Tween vs. saline) in the control animals in respective parts of the experiment (see Method). Importantly, the within group variability of data appeared to be low as indicated by the SEM.

One of the most interesting findings of the present study was a lack of an immediate effect of buspirone and its delayed anxiolytic-like action, at the doses of 1.2 mg/kg and 2.4 mg/kg. A number of studies have shown the anxiolytic effects of 5-HT_{1A} receptor agonists at in humans (9,11,44), and in some animal models of anxiety (22,35,41). The direct antiemotional prop-

TABLE 2

OPEN FIELD TEST—POST-TEST INJECTIONS

Group	Motor Activity Day 1	Motor Activity Day 2	Entries Into Central Sector—Day 1	Entries Into Central Sector—Day 2	Anti-Thigmotactic Effect—Day 1	Anti-Thigmotactic Effect—Day 2
Control (1% Tween)	89.3 ± 10.3	69.7 ± 8.0##	9.2 ± 2.0	8.9 ± 1.8	88.1 ± 18.4	119.0 ± 19.7
Diazepam 1.5 mg	95.2 ± 5.2	55.2 ± 6.3##	11.3 ± 1.9	5.2 ± 1.3#	113.2 ± 16.1	94.6 ± 19.1
Control (1% Tween)	83.6 ± 8.7	56.4 ± 12.3##	9.7 ± 1.4	6.6 ± 2.2	119.0 ± 14.2	97.6 ± 25.5
Zolpidem 1.0 mg	94.1 ± 8.1	54.6 ± 5.9##	9.3 ± 1.2	6.7 ± 1.1#	106.7 ± 12.7	118.4 ± 17.5
Control (saline)	77.5 ± 7.7	34.0 ± 6.5##	4.2 ± 0.9	1.1 ± 0.3#	51.2 ± 10.8	51.9 ± 19.5
Buspirone 2.4 mg	64.1 ± 6.8	33.4 ± 7.0##	2.6 ± 1.0	0.8 ± 0.4	34.7 ± 11.7	15.5 ± 6.7

Motor activity, the number of central entries and anti-thigmotactic effect (calculated as a ratio of the number of entries into the central part of the open field to the rat locomotor activity, and multiplied by 1000). The anti-thigmotactic ratio was calculated for each rat separately and then the mean value for each experimental group was received. The drugs were injected IP immediately after the first session in the open field. The number of rats in each experimental group varied from 9 to 10. The data are shown as means ± SEM, "#" = day-to-day, within group comparisons. # = $p < 0.05$; ## = $p < 0.01$.

TABLE 3
BRAIN [^{3}H]MUSCIMOL BINDING

Brain Structure	Control	Buspirone	p	% Ratio
Frontal cortex	3.826 ± 0.15	4.345 ± 0.18	0.04	(+) 13.6
Dentate gyrus	2.964 ± 0.13	3.383 ± 0.18	0.08	(+) 14.1
Nucleus accumbens	2.219 ± 0.12	2.481 ± 0.12	0.14	(+) 11.8
Caudate putamen	1.659 ± 0.12	1.850 ± 0.09	0.23	(+) 11.5
Dorsal raphe nucleus	1.619 ± 0.14	1.619 ± 0.12	1.00	0.0

Specific binding of [^{3}H]muscimol to the $GABA_A$ receptors in different brain structures after pretreatment of rats with buspirone at the dose of 2.4 mg/kg IP. The data are shown as means ± SEM in nCi/mg tissue. The number of rats in each experimental group varied from 6 to 10.

erties of buspirone were also confirmed in this study. Interestingly, it was also revealed that when buspirone was injected at the dose of 1.2 and 2.4 mg/kg before the test, it significantly lowered the thigmotaxis parameter on the second test session 24 h later. The above data remain in line with previous clinical reports of delayed action of buspirone (6,25). In comparison, diazepam and zolpidem failed to reveal similar delayed in time anxiolytic potential. Although it is difficult to compare clinical data with the effects of acute treatment of rats with buspirone in the animal model of anxiety, the results presented here confirm a unique pattern of a psychopharmacologic profile of this drug.

The mechanism for the delayed anxiolytic action of buspirone may be related to a phenomenon of time-dependent sensitization, i.e., a response growing with the passage of time after a single triggering dose of a drug, and continuing even when the drug is completely washed out of the organism (2). A number of preclinical and clinical studies have focused on research with antidepressants in which acute pulse (high) doses of a drug resulted in the outcome comparable to that produced by a daily treatment (1,21,24,31). Also in case of the delayed action of buspirone, reported in the present experiment, the mechanism of a time-dependent sensitization may be taken into account, as a short half-life of this compound (about 3 h) can not explain the effects observed on the second day. However, due to paucity of experimental data, apparently more research at different time-points is needed before any firm conclusion about a mechanism of the delayed effect of buspirone can be drawn.

In this context, it is noteworthy that many behavioral and physiologic stressors and fear-evoking stimuli were found to augment 5-HT output and turnover within different rat forebrain structures (8,17,33). Serotonin is implicated in the control of neural processes activated by fear-evoking conditions. Moreover, 5-HT depletion seems to be specifically related to anxiety reduction (cf. 16), which holds true also in case of acute administration of buspirone, if one considers an inhibiting output of presynaptic $5\text{-}HT_{1A}$ receptors on 5-HT neurons activity (27). Benzodiazepines are likewise considered to decrease 5-HT turnover; however, this effect has been found to depend on the dose of the drug and the brain structure examined (14). Recently, it was reported that different $GABA_A$ receptor complex ligands produced both an anticonflict effect and decreased 5-HT turnover in the rat forebrain structures (26). The $5\text{-}HT_{1A}$ receptor is thought to be involved in this phenomenon, as it was recently found that repeated diazepam administration increased the density and affinity of $5\text{-}HT_{1A}$ receptors in rat midbrain (34). Serotonin depletion due to pretreatment with a serotonin synthesis inhibitor, p-CPA, resulted in increase in [^{3}H]zolpidem binding to the rat occipital cortex (26). Similar increase in binding to $GABA_{A1a}$ receptors in the rat substantia nigra was reported in the study of Gobbi et al. (10) in which binding of [^{3}H]flumazenil in the presence of triazolopiridazine was evaluated following chronic buspirone administration. Accordingly, the biochemical results obtained in the present study, i.e., the significant enhancement in [^{3}H]muscimol binding after a single dose of buspirone within the frontal cortex, and a similar, near-significant, tendency in the dentate gyrus of the hippocampus indicate that a reduced 5-HT turnover (due to buspirone pretreatment) is accompanied by a disinhibition of GABA system activity in some brain structures. It was recently suggested that the inhibition of 5-HT activity, either due to neurotoxin treatment or $5\text{-}HT_{1A}$ receptor agonist administration, may involve the release of positive modulators of the $GABA_A$/BDZ receptor complex (40). Thus in this way the hypoactivity of 5-HT system could interact synergistically with a direct anticonflict effect of benzodiazepine receptor ligands. Furthermore, the localization of the revealed biochemical changes stays in line with the knowledge about a critical role the forebrain cortical and limbic areas are playing in rat reaction to novelty (4,5,18), and in the central effects of all clinically recognized anxiolytics (12,13). The fact that buspirone was given only once at the moderate dose can probably explain the weak, but still significant effect of the drug on [^{3}H]muscimol binding. It can be assumed that initiation of changes in 5-HT/GABA balance after buspirone injections on the first day brings about a significant modification in animals emotional behavior, examined on the following day.

In conclusion, the presented study provides more experimental data validating buspirone as an important drug devoid of disruptive influence on motor and cognitive processes, and useful in the treatment of anxiety-related disorders. The drug appears to exhibit the most "clear" anxiolytic-like profile of action in the open field test as modified by us. This part of the data is of special interest, because in other tests (elevated plus maze, the Vogel test, aversive brain stimulation) buspirone, widely used in clinical practice, yielded disparate results. Thus the open field test emerges as a simple and sensitive tool to differentiate the activity spectra of ligands at the $GABA_A$ receptor complex and $5\text{-}HT_{1A}$ receptors, confirming their clinical profiles of activity. Finally, the buspirone-induced changes in the binding of [^{3}H] muscimol to brain structures shed some light on the manner in which 5-HT/GABA interaction may operate in controlling emotional behavior.

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