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Behavioral Effects of $GABA_A$ Receptor Stimulation and GABA-Transporter Inhibition

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SCHMITT, U., H. LÜDDENS, AND C. HIEMKE. *Behavioral effects of $GABA_A$ receptor stimulation and GABA-transporter inhibition*. PHARMACOL BIOCHEM BEHAV **65**(2) 351–356, 2000.—The present analysis addressed behavioral changes after treatment with 4.5 mg/kg or 18.5 mg/kg of the GABA-uptake inhibitor tiagabine combined with either the benzodiazepine diazepam (1.5 mg/kg) or the imidazopyridine zolpidem (0.05 mg/kg), the latter two acting differentially on $GABA_A$ receptor subtypes. The study included 97 male PVG/OIaHsd rats. A standard open field, an enriched open field, and an elevated plus-maze was used to study rat behavior. Treatment with the low dose of tiagabine alone induced no specific behavioral effects, whereas the high dose had an anxiolytic-like potential. Furthermore, diazepam but not zolpidem displayed anxiolytic-like effects. Combination of each benzodiazepine receptor agonist with tiagabine at the low dose decreased explorative activity. Diazepam plus the high dose of tiagabine increased the activity in the open-field test. Zolpidem together with 18.5 mg/kg tiagabine had an angiogenic-like effect compared to pure tiagabine treatment. These results provide evidence for a pharmacodynamic interaction between the GABA-uptake inhibitor tiagabine and diazepam or zolpidem. The interaction might be relevant in the clinic when combining the anticonvulsant tiagabine and a benzodiazepine receptor agonist. © 2000 Elsevier Science Inc.

Rat behavior GABA transporter Benzodiazepines Anxiety Tiagabine Zolpidem Diazepam

GAMMA-AMINO BUTYRIC ACID (GABA) is the major inhibitory neurotransmitter in the brain. Modulation of its transmission through $GABA_A$ receptors by a benzodiazepine (BZ) receptor ligand results in sedation, amnesia, muscle relaxation, and anxiolysis [for review see (21)]. Benzodiazepines (BZs) are, therefore, frequently used in the treatment of anxiety disorders (37,41). They act through specific BZ recognition sites integral to $GABA_A$ receptors by increasing the frequency of the channel opening (27). $GABA_A$ receptors are pentameric, membrane-associated glycoproteins (25) that assemble from the 14 different receptor subunits that have been so far identified in mammals (α_1–α_6, β_1–β_3, γ_1–γ_3, δ, and ϵ). The α_1-subunit, together with $\beta x \gamma_2$, confers the classical BZ-type I (BZ1) pharmacology (11), whereas α_2-, α_3-, or α_5-subunit composition results in a BZ-type II-like (BZ2) pharmacology in receptors additionally containing any β variant and the γ_2 subunit (32). In contrast to diazepam, the imidazopyridine zolpidem displays only a high affinity and efficacy at the BZ1 binding site, but low affinity at BZ2 binding sites (23). The presence of the $\alpha 5$ polypeptide yields receptors with extremely low affinity for zolpidem. Likewise, all recombinant receptors containing the γ_3 subunit are insensitive to zolpidem. BZ binding site recognizing ligands, however, bear the risk of tolerance and dependence in animals and in humans (22).

In addition to BZ site ligands, GABAergic transmission can be enhanced by inhibiting GABA transporters (GAT), which remove GABA from the synaptic cleft (19). So far, four distinct rat GATs (GAT-1 to GAT-4) have been identified and cloned (5,12,17,19,26). Inhibition of GABA uptake may have advantages over neuronal stimulation by direct $GABA_A$ receptor agonists or BZs, as it will prolong the effect of endogenously released GABA, thus retaining the physiological specificity. Furthermore, the extent of the enhancement of $GABA_A$ receptor-mediated function by a GABA uptake inhibitor will be limited by the amount of GABA released. Beyond their anticonvulsant effects the behavioral effects of GAT-ligands are poorly characterized (4,5,40). Modifications of activity and exploration have been reported for high doses of GAT-inhibitors (8,39) and an anxiolytic-like potential of GAT-inhibitors has been concluded from conflict paradigms (1,14,30). Dalvi and Rodgers (10) reported weak anxiolytic-like effects of a GAT-inhibitor in the elevated plus-

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maze test for anxiety. Our own data supports this potential of GAT-inhibitors (35).

The purpose of this study was to evaluate the effects of concomitant $GABA_A$ receptor stimulation and GAT inhibition on rat behavior. We used tiagabine (TGB), a GAT-inhibitor with high affinity to GAT-1 and moderate affinity to GAT-2 and GAT-3 in rats (5) alone or together with either zolpidem or diazepam as agonists at the $GABA_A$ receptor BZ-binding sites. We used two open-field tests and the elevated plus-maze to characterize activity, exploration, and anxiety after acute treatment (36).

METHODS

Animals

Adult male PVG/OIaHsd (PVG) rats (150–200 g) obtained from Harlan–Winkelmann (Borchen, Germany) were housed four per cage at 22°C, a relative humidity of 60% and a 12 L:12 D cycle (light on from 0600 until 1800 h). Food and water were provided ad lib.

Drugs

All drugs were dissolved in physiological saline (0.9%) and given the following doses: Tiagabine (donated by Novo Nordisk, Copenhagen, Denmark): 4.5 and 18.5 mg/kg; diazepam (provided by Hoffman–LaRoche, Basel, Switzerland) 1.5 mg/kg; zolpidem (donated by Synthelabo, Paris, France) 0.05 mg/kg. Pure saline was used as control substance. All drugs were injected IP in a volume of 2.5 ml. Drug combinations were injected simultaneously in the same volume.

Experimental Design and Testing Routine

A total of 97 rats were divided into nine groups: first, control ($n = 11$); second and third, tiagabine 4.5 or 18.5 mg/kg ($n = 11$ and $n = 10$); fourth, zolpidem 0.05 mg/kg ($n = 11$); fifth and sixth, zolpidem plus tiagabine in any of the two concentrations ($n = 11$ each); seventh, diazepam 1.5 mg/kg ($n = 10$); eighth and ninth, diazepam plus tiagabine in any of the two concentrations ($n = 11$ each). All animals received habituation handling for 1 week 5 min daily before commencing the tests (36). Tests started at day 1 with the standard open field (10 min), followed on day 4 by the enriched open field (10 min) and on day 7 the rats were tested in the elevated plus-maze (7.5 min). All tests were carried out between 0900 and 1300 h. Rats were transported within their home cages to the test room at least 1 h before commencing the test. Drugs were injected 30 min before testing. After each trial, the test arenas were carefully cleaned.

The experimental protocols met the guidelines of the German law on animal studies and were approved by the Bezirksregierung Rheinhessen-Pfalz (Az 177-07/941-22).

Instrumentation and Behavioral Tests

Open field. The open-field test arena consisted of dark-gray plastic with the bottom painted in ochre. It was divided into 25 squares (A1 and E5) by gray lines. The indirectly illuminated arena measured 100 × 100 × 35 cm. The following parameters were recorded: total distance moved (cm), number of entries into the center (n), number of rears (n).

Enriched open field. The open-field arena was used to test animal behavior in an enriched environment by introduction of two novel objects of ~9 cm and ~14 cm in diameter. The objects were placed on squares B2 and D3 of the arena. In this arrangement the following data were recorded: total distance moved (cm), approaches to novel objects (n), maximum time spent during one visit at the object (s), time spent on objects (percent of recording time) and the number of rears (n).

Elevated plus-maze. The plus-maze was made of the same material as the open-field arena. It consisted of two open arms 42.5 × 15 cm, and two enclosed arms 42.5 × 15 × 14 cm. The arms extended from a central platform, 15 × 15 cm. The apparatus was mounted on a metal frame 70 cm above the floor. The video camera and the illumination lamps were attached to the metal frame. The parameters recorded for the elevated plus-maze were total distance moved (cm), closed arm entries (n), % time on open arms (percent of recording time), open-arm entries (percent of total and number, n). In addition, the number of head dips (HD, n) and closed arm returns (CAR, n) were counted.

Monitoring of Behavior

For all test sessions the path of each rat was registered automatically by a computerized image analysis system. The hardware consisted of an IBM-type AT computer combined with a videodigitizer and a CCD video camera. The software used for data acquisition and analysis was EthoVision (Noldus Information Technology, Utrecht, The Netherlands). Head dips and closed-arm returns were counted by an observer blind to group assignment of the rats from a video tape of the tracks.

Statistical Analysis

Data were analyzed by two factor analysis of variance (two-way ANOVA; first factor benzodiazepine, second factor GAT inhibition). Post hoc comparisons were made when appropriate with the Tukey test for unequal n. Differences were considered as statistically significant for $p < 0.05$.

RESULTS

Effects of Zolpidem of Diazepam Treatment on Rat Behavior

Tables 1 and 2 summarize the data and ANOVA statistics (factor 1) of the effects of 0.05 mg/kg zolpidem and 1.5 mg/kg diazepam. Zolpidem had weak effects on activity, which was significantly decreased only in the enriched open-field test (Table 1). It decreased the exploration activity, as indicated by a significant decrease in the percent time spent at the novel objects as well as a decrease in the number of visits. In parallel, closed arm returns (CAR) were increased and the center activity was, by trend, decreased (Table 1). Effects on anxiety were minor as only one of three parameters was decreased (percent time on open arm). Diazepam (1.5 mg/kg) had no effects on activity and on exploration with the exception of an increase in the number of rears in the open field (Table 2). In the elevated plus-maze test the anxiety-related parameters on open-arm entries were significantly increased (Table 2).

Effects of Tiagabine Treatment on Rat Behavior

Data and ANOVA statistics (factor 2) on TGB effects are summarized in Tables 1 and 2. All TGB effects were dose dependent, at 4.5 mg/kg no statistically significant changes in rat behavior occurred. At the high-dose treatment (18.5 mg/kg) TGB had no effect on activity related parameters, i.e., distance moved and closed arm entries in any of the tests (Tables 1 or 2). Exploration-related parameters were significantly affected, evidenced by a nearly three-fold increase in center ac-

TABLE 1

DRUG EFFECTS ON RAT BEHAVIOR AFTER ACUTE TIAGABINE (TGB) TREATMENT (4.5 AND 18.5 mg/kg) OR ACUTE TREATMENT WITH ZOLPIDEM (ZOL; 0.05 mg/kg) AND IN COMBINATION WITH TIAGABINE IN AN OPEN FIELD, AN ENRICHED OPEN FIELD, AND AN ELEVATED PLUS-MAZE

Parameter	Control	TGB 4.5 mg/kg	TGB 18.5 mg/kg	Zol 0.05 mg/kg	ZolTGB 4.5 mg/kg	ZolTGB 18.5 mg/kg	Factor1 $F(1, 59)$ Factor2 $F(2, 59)$	Factor1×2 $F(2,59)$
				Open-Field Behavior				
Distance moved	1807 ± 280	1795 ± 364	891 ± 255	1808 ± 244	1759 ± 283	1743 ± 372	1.19 NS 1.59 NS	1.33 NS
Entries in center	5.7 ± 1.5	3.9 ± 0.9	16.8*‡ ± 3.2	3.9 ± 0.9	3.1 ± 0.6	9.0 ± 3.7	4.00 NS 11.22 $p < 0.001$	1.54 NS
Rears	1.8 ± 0.4	1.5 ± 0.4	0.2*‡ ± 0.1	2.8 ± 0.9	2.4 ± 0.7	0.5 ± 0.2	2.83 NS 6.93 $p < 0.01$	0.17 NS
				Enriched Open-Field Behavior				
Distance moved	1647 ± 150	2262 ± 340	1789 ± 394	1103* ± 306	979 ± 283	1754 ± 352	5.96 $p < 0.05$ 0.82 NS	2.02 NS
Max Obj.	11.7 ± 1.2	15.3 ± 2.9	26.6* ± 7.8	8.3 ± 1.4	13.1 ± 4.6	15.0 ± 3.3	3.03 NS 3.61 $p < 0.05$	0.79 NS
n Obj.	12.2 ± 1.3	15.8 ± 2.5	10.4 ± 1.9	7.1* ± 2.7	5.6 ± 1.9	9.7 ± 2.9	7.99 $p < 0.01$ 0.12 NS	2.12 NS
% t Obj.	10.3 ± 1.1	13.6 ± 2.7	16.7* ± 4.0	5.0* ± 1.6	5.8 ± 1.8	11.4 ± 3.2	8.53 $p < 0.01$ 3.20 $p < 0.05$	0.17 NS
Rears	0.36 ± 0.2	0.73 ± 0.2	0.0 ± 0.0	0.64 ± 0.3	0.27 ± 0.2	0.0 ± 0.0	0.18 NS 5.38 $p < 0.01$	2.25 NS
				Plus-Maze Behavior				
Distance moved	923 ± 129	872 ± 118	994 ± 171	746 ± 110	750 ± 105	818 ± 145	2.20 NS 0.28 NS	0.03 NS
Closed entries	10.7 ± 2.1	10.0 ± 1.4	7.7 ± 1.2	7.5 ± 1.5	9.1 ± 2.1	8.8 ± 2.1	0.47 NS 0.27 NS	0.73 NS
Open entries	5.6 ± 1.0	4.6 ± 1.8	13.1‡ ± 3.1	5.6 ± 1.3	4.5 ± 1.6	5.8 ± 1.8	2.81 NS 4.02 $p < 0.05$	2.57 NS
% open entries	35.9 ± 5.1	24.6 ± 6.3	61.6‡ ± 8.3	39.7 ± 5.7	28.8 ± 4.3	31.9† ± 6.3	2.10 NS 5.39 $p < 0.01$	4.97 $p < 0.05$
% t on open arms	6.8 ± 2.1	4.2 ± 1.4	44.5*‡ ± 10.4	2.6* ± 0.6	1.7 ± 0.5	10.7† ± 5.5	12.63 $p < 0.001$ 17.17 $p < 0.001$	7.06 $p < 0.01$
HD	8.5 ± 1.0	9.6 ± 1.2	14.3* ± 2.8	6.7 ± 1.0	7.4 ± 1.3	11.0 ± 2.6	2.70 NS 4.50 $p < 0.05$	0.10 NS
CAR	0.6 ± 0.2	1.2 ± 0.3	0.6 ± 0.3	1.1* ± 0.3	1.6 ± 0.4	1.5 ± 0.4	6.02 $p < 0.05$ 1.70 NS	0.32 NS

Data presented are mean values ± SEM of 10 to 11 animals. Significant post hoc results with $p < 0.05$ are indicated by: *Compared to control, †interaction, ‡compared to different dose.

tivity in the open field and a 60% increase in the percent time spent at the novel objects. These increases are in line with the changes in the maximal time spent at the objects. Also, in the elevated plus-maze the head dips were increased significantly (Tables 1 or 2). As well, TGB treatment at the dose of 18.5 mg/kg significantly increased open-arm activity in the elevated plus-maze, the anxiety-related parameters (Tables 1 or 2).

Interactive Effects of Coapplication of Zolpidem and Tiagabine on Rat Behavior

Data and ANOVA statistics (factors 1×2) on zolpidem and TGB effects are summarized in Table 1. The low dose of TGB (4.5 mg/kg) together with zolpidem showed the same pattern as zolpidem treatment alone (Table 1). The high dose of TGB (18.5 mg/kg) administered together with zolpidem resulted in a behavior similar to control conditions, i.e., the anxiety-reducing potency of TGB in the elevated pluz-maze was significantly counteracted by zolpidem (Table 1).

Interactive Effects of Coapplication of Diazepam and Tiagabine on Rat Behavior

Data and corresponding ANOVA statistics (factors 1×2) are summarized in Table 2. No interaction was seen by the combination of the low dose of TGB with diazepam in any parameter related to activity or exploration. However, there was an obvious trend towards an increase in all anxiety-related parameters with a significant interaction in the percent open-arm entries. Treatment with the high dose of TGB and diazepam resulted in a significantly increased activity in both open-field tests. In addition, center activity (by trend) and novel-object exploration increased significantly (number of visits; Table 2). In the

TABLE 2

DRUG EFFECTS ON RAT BEHAVIOR AFTER ACUTE TIAGABINE (TGB) TREATMENT (4.5 AND 18.5 mg/kg) OR ACUTE TREATMENT WITH DIAZEPAM (DIA; 1.5 mg/kg) AND IN COMBINATION WITH TIAGABINE IN AN OPEN FIELD, AN ENRICHED OPEN FIELD, AND AN ELEVATED PLUS-MAZE

Parameter	Control	TGB 4.5 mg/kg	TGB 18.5 mg/kg	Dia 1.5 mg/kg	DiaTGB 4.5 mg/kg	DiaTGB 18.5 mg/kg	Factor1 $F(1, 58)$ Factor2 $F(2, 58)$	Factor1×2 $F(2, 58)$
				Open-Field Behavior				
Distance moved	1807 ± 280	1795 ± 364	891 ± 255	1861 ± 426	1986 ± 227	4216*† ± 691	12.65 $p < 0.001$ 1.98 NS	10.11 $p < 0.001$
Entries in center	5.7 ± 1.5	3.9 ± 0.9	16.8*‡ ± 3.2	5.3 ± 1.2	4.3 ± 0.9	12.0 ± 2.0	1.29 NS 20.28 $p < 0.001$	1.26 NS
Rears	1.8 ± 0.4	1.5 ± 0.4	0.2* ± 0.1	3.8* ± 1.3	1.6 ± 0.4	1.2 ± 0.5	4.25 $p < 0.05$ 5.76 $p < 0.01$	1.05 NS
				Enriched Open-Field Behavior				
Distance moved	1647 ± 150	2262 ± 340	1789 ± 394	1202 ± 318	1097 ± 302	2726† ± 560	0.56 NS 2.68 NS	4.27 $p < 0.05$
Max Obj.	11.7 ± 1.2	15.3 ± 2.9	26.6*‡ ± 7.8	8.3 ± 2.3	9.5 ± 1.7	24.1 ± 5.7	1.29 NS 7.61 $p < 0.01$	0.90 NS
n Obj.	12.2 ± 1.3	15.8 ± 2.5	10.4 ± 1.9	7.9 ± 2.6	7.5 ± 2.6	18.4† ± 3.7	0.53 NS 1.42 NS	5.37 $p < 0.01$
% t Obj.	10.3 ± 1.1	13.6 ± 2.7	16.7*§ ± 4.0	5.2 ± 1.7	5.3 ± 1.6	20.6 ± 5.2	1.54 NS 7.07 $p < 0.01$	2.11 NS
Rears	0.36 ± 0.2	0.73 ± 0.2	0.0 ± 0.0	0.1 ± 0.1	0.36 ± 0.2	0.27† ± 0.1	0.90 NS 4.00 $p < 0.05$	2.51 NS
				Plus-Maze Behavior				
Distance moved	923 ± 129	872 ± 118	994 ± 171	735 ± 144	876 ± 121	1258 ± 135	0.06 NS 2.73NS	1.37 NS
Closed entries	10.7 ± 2.1	10.0 ± 1.4	7.7 ± 1.2	6.0 ± 1.5	9.6 ± 1.7	11.7 ± 2.5	0.06 NS 0.40 NS	2.87 NS
Open entries	5.6 ± 1.0	4.6 ± 1.8	13.1* ± 3.1	7.9* ± 1.9	14.4 ± 4.4	15.4 ± 2.9	4.51 $p < 0.05$ 3.73 $p < 0.05$	1.22 NS
% open entries	35.9 ± 5.1	24.6 ± 6.3	61.6‡ ± 8.3	55.9* ± 6.3	58.8† ± 6.9	53.5 ± 6.4	6.22 $p < 0.05$ 3.81 $p < 0.05$	4.54 $p < 0.05$
% *t* on open arms	6.8 ± 2.1	4.2 ± 1.4	44.5*‡ ± 10.4	13.9 ± 6.6	14.7 ± 5.8	28.6 ± 7.4	0.10 NS 12.09 $p < 0.001$	2.64 NS
HD	8.5 ± 1.0	9.6 ± 1.2	14.3*‡ ± 2.8	8.5 ± 1.7	12.2 ± 1.6	18.5 ± 2.8	2.00 NS 8.33 $p < 0.001$	0.55 NS
CAR	0.6 ± 0.2	1.2 ± 0.3	0.6 ± 0.3	0.2 ± 0.1	0.6 ± 0.2	0.7 ± 0.3	1.74 NS 1.75 NS	1.06 NS

Data presented are mean values ± SEM of 10 to 11 animals. Significant post hoc results with $p < 0.05$ are indicated by: *compared to control, †interaction, ‡compared to different dose.

elevated plus-maze, no significant changes occurred, the behavior was almost similar to either of both mono treatments (Table 2).

DISCUSSION

Inhibition of the GABA Transport by Tiagabine

Blockade of GABA uptake by inhibiting GABA transporters (GAT) increases extracellular GABA in the synaptic cleft (19). The two doses of TGB (4.5 and 18.5 mg/kg) used in the present investigation increase the concentrations of extracellular GABA (13) and alter rat behavior (35). Our results indicate that augmented extracellular GABA induced dose-dependent behavioral changes. Explorative activity is enhanced indicated in the enriched open field by an increase in the maximum time spent at the novel objects (the high dose of TGB), a finding that parallels previously observed diazepam effects (34). A similar enhancement of exploration is seen in the standard open field and the elevated plus-maze. The trend of the high dose TGB to decrease locomotion-related parameters in the open field can be due to sedation, as known for high doses of benzodiazepines (BZ), or due to the anticonvulsant, muscle relaxing properties of the drug observed at doses >30 mg/kg (5,22). However, because the doses were lower than this concentration, the latter explanation seemed unlikely (18,39).

In the elevated plus-maze test TGB (18.5 mg/kg) exhibited no effect on activity, whereas parameters related to anxiety (31) displayed an anxiolytic-like effect. Comparable changes in anxiety-related behaviors have been reported after enhancing GABAergic transmission by administration of diazepam or other BZs (2,20,28,38). Moreover, the anxiolytic-like profile of TGB is similar to that of diazepam in the elevated plus-maze test (9). Present results were supported by Dalvi and Rodgers (10) showing, besides the known effects of diazepam, an enhancement of behaviors related to risk assessment of

NO-711, an other GAT inhibitor. Although NO-711 exerts only weak anxiolytic-like properties, these results provide additional evidence for the anxiolytic-like potential of GAT inhibitors as obvious by the present results.

$GABA_A$ Receptor Facilitation, Transporter Inhibition, and Possible Drug Interaction

The present results confirm the anxiolytic-like effects of diazepam, most prominent in the elevated plus-maze test (3,16,20). The smaller effects compared to previously published data can be explained by the lower dose (34) chosen to allow for possible additive effects of TGB (14). A similar situation was expected in the case of zolpidem, which at 0.05 mg/kg has only minor effects on rat behavior [(16,34; Table 1). We assumed that increasing the amount of GABA in the synaptic cleft by TGB might enhance the effects of zolpidem (14–16). However, combining zolpidem with 4.5 mg/kg TGB did not change the rat behavior, but zolpidem abolished all effects of pure TGB when given together with the high dose of TGB. This effect could be interpreted as an angiogenic-like action of zolpidem at high GABA concentrations in the synaptic cleft. This contrasts with effects of diazepam. The combination of diazepam and TGB (4.5 mg/kg) showed a trend towards an increase in the anxiolytic-like potential of the combination compared to diazepam alone. Detailed statistical analysis confirmed that the significant increase in the parameter percent open-arm entries compared to control level was due to a specific drug interaction of the combination treatment. In parallel, the high dose of TGB and diazepam failed to further modify the anxiety-related behaviors compared to the pure treatment. On one hand, the lack of a statistically significant additivity of both, and especially the high dose, might be caused by a ceiling effect implemented in the test paradigm, which requires an exploration of the whole maze including the closed arms. On the other hand, it could be explained by the fact that BZs have no further effect at $GABA_A$ receptors at saturating concentrations of GABA, which could be the case after treatment with 18.5 mg/kg TGB. However, the same combination of 18.5 mg/kg TGB and diazepam resulted in a significantly increased overall activity in both open-field tests, which argues against the saturation hypothesis. Thus, this paradoxical effect cannot be explained by simple additive effects but requires a specific drug interaction that might be test dependent as well as dependent on the $GABA_A$ receptor pharmacology.

The observed behavioral changes confirmed the assumption that increased GABA concentrations in the synaptic cleft by inhibition of the GABA reuptake affects responses to BZ receptor ligands. Interestingly, in the case of anxiety-related behavior, the direction of modulation by combined treatment of BZ receptor ligands and GAT-inhibitors seemed to be dependent on the BZ receptor pharmacology. Pure BZ1 type pharmacology resulted in an inverse agonistic effect with respect to TGB, whereas unselective BZ pharmacology showed small but additive effects. This differences could result from different molecular mechanisms, i.e., the addition of independent single drug effects or be due to a specific drug-drug interaction (14). Those drug-dependent differences in the efficacy of a GAT-inhibitor to potentiate $GABA_A$ receptor-induced behaviors were so reported by Giusty et al. (14,15). Furthermore, it can be a result of a stimulus-dependent (i.e., the behavioral test) $GABA_A$ receptor subtype specific modulation of behavior. There is increasing evidence that the subtype composition of $GABA_A$ receptors can be related to certain behaviors (33,34). Because of that, it is conceivable that situation-dependent distinct $GABA_A$ receptor subtypes are preferentially activated. This, under the present pharmacological conditions, could mean that if the more general increase in GABAergic tone due to GAT inhibition support the subtype specific activation, it may result in additive effects, i.e., diazepam and tiagabine in the anxiety-related behavior (plus-maze) as well as effects on activity and exploration (open field). Otherwise, if the subtype specific activation counteracts the stimulus-dependent activation, it may result in unforeseen interaction effects, i.e., zolpidem and tiagabine as the α_1-subunit is not related to anxiety (33). Thus, the differential modification of rat behavior after administration of BZ-site ligands together with GAT inhibitors indicates that the interaction deserves further evaluation of the molecular mechanisms and the role of $GABA_A$ receptor composition.

To our knowledge, no data exists that explored the interaction of GAT inhibitors and $GABA_B$-receptor ligands. However, it can be assumed that GAT inhibition results, due to an increase in extracellular GABA, in effects similar to that of $GABA_B$-receptor agonists. With respect to locomotion and exploration in an open-field test, Car and Wisniewski (7) reported no effects of the $GABA_B$ agonist baclofen, and Dalvi and Rodgers (10) did not find evidence linking $GABA_B$ receptor function to anxiety. On the other hand, these latter authors reported reduced activity after treatment with the $GABA_B$ agonist baclofen on the elevated plus-maze. $GABA_B$ receptors generally are suggested to play a role in mediating antidepressant effects, but seem not to influence exploratory behavior or risk assessment (6,7,24,29).

Our results might be of relevance in a clinical setting. Because of different indications, tiagabine and benzodiazepines may be prescribed concomitantly without being aware of possible pharmacodynamic interactions. Because effects of combining tiagabine and benzodiazepines so far have not been controlled in patients or healthy subjects, our results indicate that patients should be carefully supervised when a combination treatment of tiagabine and a benzodiazepine agonist is used.

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