Pharmacology Biochemistry and Behavior, Vol. 53, No. 2, pp. 271–275, 1996

0091-3057/96 $15.00 + .00

0091-3057(95)02001-P

Anxiolytic-Like Effect of Combined Extracts of *Zingiber Officinale* and *Ginkgo Biloba* in the Elevated Plus-Maze

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Received 7 February 1995

HASENÖHRL, R. U., CH. NICHAU, CH. FRISCH, M. A. DE SOUZA SILVA, J. P. HUSTON, C. M. MATTERN AND R. HÄCKER. *Anxiolytic-like effect of combined extracts of* zingiber officinale *and* ginkgo biloba *in the elevated plus-maze.* PHARMACOL BIOCHEM BEHAV **53**(2) 271–275, 1996.—The effects of the known anxiolytic compound diazepam (DZ) on the behavior of rats in the elevated plus-maze were compared with those of zingicomb (ZC) (registered trademark of Mattern et Partner), a combination preparation of standardized extracts of *ginkgo biloba* and *zingiber officinale*. DZ was administered intraperitoneally (IP) in a reference dosage of 1 mg/kg 30 min before the rats were tested on the elevated plus-maze for 5 min. The treatment with DZ elevated the time spent on the open arms and excursions into the end of the open arms, increased scanning over the edge of an open arm, and decreased risk-assessment from an enclosed arm. ZC was administered intragastrically (IG) in four doses ranging between 0.5 and 100 mg/kg 60 min prior to plus-maze testing. The treatment with 0.5 mg/kg ZC elevated the time spent on the open arms and excursions into the end of the open arms; at the high dosage of 100 mg/kg, ZC led to fewer excursions to and less scanning of the open arms. Injection of 1 or 10 mg/kg ZC had no significant effect on the behavior in the maze. These data provide evidence that ZC has anxiolytic effects in the elevated plus-maze comparable to those of DZ, but that in high dosage the phytopharmacon may also have anxiogenic properties. The anxiolytic-like effects of ZC are discussed with regard to the known antiserotonergic action of ginger and *ginkgo biloba*.

Elevated plus-maze Anxiety Diazepam *Ginkgo biloba* Ginger Anxiolytics Anxiogenic drugs Serotonin 5-HT_3 receptor Rat

SEROTONERGIC drugs are receiving much attention as potential anxiolytic agents in the search for alternatives to the benzodiazepines (19,25). The hypothesis that anxiety is increased by excessive serotonin (5-HT) and decreased by a reduction of 5-HT activity (22) is supported by several findings. Benzodiazepines (BZDs) reduce the activity of 5-HT in the brain (31). Selective 5-HT_1 receptor agonists, for example RU-24969 (8), have anxiogenic effects, whereas 5-HT depletion with *p*-CPA (9) and 5,7-DHT (2), or blockade of the 5-HT_2 receptor subtype, was shown to have anxiolytic effects (25). Much attention has been focused on certain 5-HT_3 antagonists, which are anxiolytic when administered peripherally or centrally (6,7,20,28). Interestingly, there is evidence that, whereas injection of BZD induces amnesia, injection of some 5-HT_3 antagonists does not (1,4,11).

Besides chemically defined drugs, there are phytopharmaca with known 5-HT antagonistic properties, such as powdered rhizomes or extracts of *zingiber officinale* and extracts from leaves of *ginkgo biloba*. Constituents of ginger, like gingerols or the diterpenoid galanolactone, are potent antagonists at the 5-HT_3 receptor (21,36). *Ginkgo biloba* extracts, in addition to their well-known action in increasing blood flow (3,23), can act as indirect serotonin-antagonists by inhibiting thrombocyte aggregation (16). Furthermore, the compound zingicomb (ZC), a combination preparation of ginger and *ginkgo biloba* extracts, was found to exert antiemetic effects in different

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animal models of emesis and in clinical trials (see Ref. 14 for a review), which were comparable to the known antiemetic action of "classical" 5-HT_3 antagonists (15,26,27).

The aim of the present study was to gauge the effect of peripheral injection of ZC on the behavior of rats in the elevated plus-maze. The plus-maze has been validated as a test for anxiety, using behavioral and physiological measures, and has shown good sensitivity to both anxiolytic and anxiogenic drugs (12,18,29,30). In the study outlined below, ZC was administered IG in four doses and its effects on exploratory activity in the plus-maze were compared with those of the known anxiolytic diazepam (DZ), which was injected systemically in a reference dosage. Zingicomb was hypothesized to have anxiolytic effects on the basis of its 5-HT_3 antagonistic properties (21,36). Because some 5-HT_3 antagonists have been reported to act as anxiolytics (6,7,20,28), it was held possible that ZC also has such effects.

METHOD

Animals

The experiments were performed on male Wistar rats (TVA, University of Düsseldorf), weighing 230–280 g at the beginning of the experiments. Rats were housed in groups of six to eight per cage, under a 12L : 12D cycle, with food and water continuously available. The experimental sessions were conducted during the rats' daylight period between 10:00 and 17:00 h. Rats were tail-marked and handled daily for 5 min during the three last days before the experiments.

Apparatus

The elevated plus-maze has been described in detail elsewhere (18,29,30). In short, it consisted of two open arms (50 × 10 cm) and two enclosed arms (50 × 10 × 40 cm) with an open roof, arranged such that the two arms of each type were opposite each other. The maze was elevated to a height of 50 cm. Illumination was provided by a 40-W red bulb suspended 150 cm above the center of the maze. Masking noise (68 dB) was provided by a noise generator. The behavior of the animals throughout the experiments was recorded by a video system. After each trial, the apparatus was swept out with water containing 0.1% acetic acid. All behavioral recordings were carried out with the observer unaware of the treatment of the rat.

Drugs and Injection Procedure

ZC, a combination preparation of standardized extracts of rhizomes of *zingiber officinale* (CO_2 extract, containing 23.5% gingerols) and *folia ginkgo biloba* (comparable to EGb 761 containing 24% ginkgoflavonglycosides), as well as DZ, were supplied by Mattern et Partner (Starnberg, Germany). The phytopharmacon was dissolved in water with the help of ultrasound, diluted to the desired concentrations with water and administrated IG via a gastric tube in a dose range from 0.5 to 100 mg/kg. The dose range of ZC was selected on the basis of pilot studies. The anxiolytic DZ (Faustan®-solution containing 18.6% ethanol) was dissolved in physiological saline (SAL) and was given IP in a dosage of 1.0 mg/kg. This dosage of DZ had repeatedly been found to be active in the elevated plus-maze (e.g., Refs. 6 and 30) and served as a reference in the present experiments. All drugs were freshly prepared before each treatment trial. The injections were given 60 min before testing (except for DZ, which was given 30 min before) in a volume of 2.0 ml/kg body weight. The same volume was used for injecting the diluent vehicles: VEH (water for ZC) and SAL (physiological saline containing two drops of ethanol for DZ).

Behavioral Procedure

Rats were randomly assigned to the following groups: SAL (n = 9) and DZ 1 mg/kg (n = 10); VEH (n = 18), ZC: 0.5 mg/kg (n = 20), 1 mg/kg (n = 20), 10 mg/kg (n = 10) and 100 mg/kg (n = 9). Each rat received an injection (IP or IG) and was then returned to its home cage. After 60 min (30 min after DZ or SAL) it was placed into the center of the plus-maze, facing one of the enclosed arms. During a 5-min test period, the following standard measures were taken for all groups: number of entries into and time spent in the open and enclosed arms, and the time spent on the central arena.

Data Analysis

Data given represent mean ± SEM values. Number of entries into and time spent on the open and enclosed arms, as well as time spent in the central arena, were analyzed either by the Student's t-test (in the case of DZ) or by one-way ANOVA (in the case of ZC). Whenever ANOVA was significant, further comparisons between vehicle- and drug-treatment groups were performed using the Duncan's new multiple range test with Kramer's modification for unequal group sizes. The level of statistical significance adopted was $p < 0.05$.

RESULTS

Rats treated with 1 mg/kg DZ spent significantly more time on the open and less time on the enclosed arms of the maze (Fig. 1); they did not differ from vehicle controls in time spent in the central arena, nor in number of entries into the open and enclosed arms as well as total arm entries. For ZC, the ANOVAs indicated a significant effect of the treatment upon the time spent on the open [$F(4, 72) = 4.79$, $p < 0.002$] as well as enclosed arms [$F(4, 72) = 6.37$, $p < 0.001$] of the maze. Posthoc analysis showed that at 0.5 mg/kg, there was a significant increase in time spent on the open arms and a significant decrease in time spent on the enclosed arms (Fig. 1); conversely, at 100 mg/kg, a decrease in time spent on the open and an increase in time spent on the enclosed arms was observed even though the respective p-values missed statistical significance. The treatment with ZC did not influence the amount of time spent in the central arena of the maze [$F(4, 72) = 1.46$, $p > 0.10$]. However, there was a significant main effect of the treatment on the number of entries into the open arms [$F(4, 72) = 4.04$, $p < 0.01$] and posthoc analysis revealed that this was due to the 10 mg/kg dosage, which significantly reduced the number of open arm entries. A significant main effect of the treatment was observed on the number of closed arm entries [$F(4, 72) = 4.54$, $p < 0.01$] and posthoc comparisons indicated a significant increase in the number of closed arm entries at 0.5 mg/kg. The treatment also significantly influenced the number of total arm entries [$F(4, 72) = 4.50$, $p < 0.01$]. Posthoc comparisons showed an increase in the number of total arm entries for rats treated with 0.5 mg/kg and a decrease for rats injected with 10 or 100 mg/kg ZC; however, the respective p-values missed statistical significance.

Other Behavioral Observations

While on the elevated plus-maze, undrugged rodents display a variety of anxiety-related behaviors in addition to the avoidance of the open arms (5,10), comprising *scanning* (protruding the head over the edge of an open arm and scrutinizing in any direction), *risk-assessment* (protruding from an en-

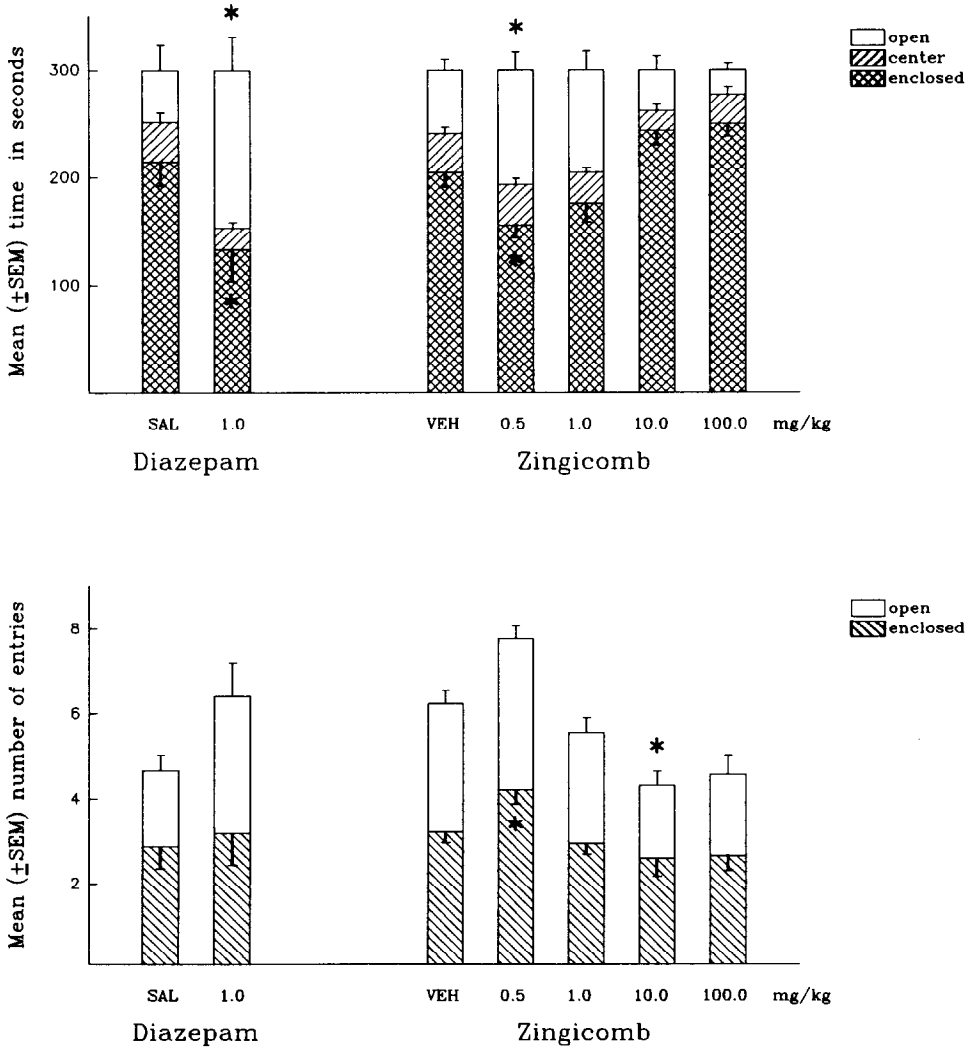

FIG. 1. Mean (±SEM) time in seconds spent in the closed arms, open arms, and central arena (above), and mean (±SEM) number of entries into the open and enclosed arms of the elevated plus-maze (below). Diazepam (DZ) was administered IP in a reference dosage of 1 mg/kg 30 min before the rats were tested on the plus-maze for 5 min. Zingicomb (ZC) was administered IG in a dose range of 0.5 to 100 mg/kg 60 min prior to the experimental session. *$p < 0.05$ drug vs. control group.

closed arm with the forepaws and head only), and *end-excursions* [number of times the rat reached the end of an open arm, a behavior that has also been dubbed "end-exploring" (10), because it is likely to reflect "exploratory" behavior]. Typically, scanning and end-excursions are decreased by anxiogenic drugs, while being increased by anxiolytics; risk-assessment is typically decreased by anxiolytic drugs (10). To examine the "anxiolytic profile" of the treatment in the present study, the frequency (*f*) and/or duration (*t*) of scanning, risk-assessment, and end-excursion were determined posthoc for the 5-min experimental session for rats injected with DZ, ZC (0.5 and 100 mg/kg), or the corresponding vehicle solutions (SAL or VEH). Between-group differences were evaluated with the Student's *t*-test and the resulting *p*-values were used as a descriptive measure of the drug-treatment effect. Rats injected with DZ showed more open arm scanning (*f:* $p = 0.020$, *t:* $p = 0.029$), less risk-assessment (*f:* $p = 0.005$, *t:* $p = 0.001$) and reached the end of the open arms more often ($p = 0.048$; Table 1). Rats treated with 0.5 mg/kg ZC reached the end of the open arms more often ($p = 0.037$), whereas scanning and risk-assessment were not influenced (*f,t:* $p > 0.05$, for both measures); rats injected with the high dosage of ZC showed fewer end-excursions ($p = 0.027$) and less scanning (*f:* $p = 0.002$, *t:* $p = 0.002$), risk-assessment was not significantly affected by the treatment (*f,t:* $p > 0.05$, for both measures).

DISCUSSION

The present results show that the phytopharmacon ZC is active in a test of fear or anxiety. For the elevated plus-maze, it has been demonstrated that the preference shown for the closed arms reflects an aversion toward the open arms, caused by fear or anxiety induced by the open space (33). ZC at 0.5

TABLE 1

FREQUENCY AND DURATION OF SCANNING, AND RISK-ASSESSMENT AND FREQUENCY OF END-EXCURSIONS FOR RATS TREATED WITH DIAZEPAM (DZ 1 mg/kg), ZINGICOMB (ZC 0.5 OR 100 mg/kg), OR WITH THE RESPECTIVE VEHICLE SOLUTION (SAL OR VEH) DURING THE 5-MIN TEST PERIOD IN THE ELEVATED PLUS-MAZE

Treatment		Scanning	Risk-Assessment	End-Excursions
Diazepam				
SAL	(*f*)	7.63 ± 3.40	14.63 ± 2.40	1.75 ± 0.77
	(*t*)	20.88 ± 9.74	56.25 ± 10.31	
DZ 1 mg	(*f*)	23.00 ± 5.49*	7.40 ± 1.19†	4.60 ± 1.29*
	(*t*)	55.80 ± 13.15*	18.80 ± 3.04†	
Zingicomb				
VEH	(*f*)	12.06 ± 1.97	13.78 ± 1.80	2.23 ± 0.51
	(*t*)	34.56 ± 5.63	41.94 ± 5.40	
ZC 0.5 mg	(*f*)	16.75 ± 3.01	12.65 ± 1.24	5.20 ± 1.22*
	(*t*)	42.95 ± 7.86	35.45 ± 5.32	
ZC 100 mg	(*f*)	2.12 ± 0.75†	11.89 ± 2.12	0.45 ± 0.24*
	(*t*)	5.89 ± 2.14†	35.89 ± 7.55	

Values are mean ± SEM; (*f*) frequency; (*t*) time in sec; *$p < 0.05$, †$p < 0.01$ drug vs. control group.

mg/kg had anxiolytic effects, as indicated by increased time spent on the open arms and increased excursions into the ends of the open arms. The compound did not influence the rats' behavior on the elevated plus-maze at doses of 1 and 10 mg/kg. Furthermore, at 100 mg/kg, injection of ZC led to fewer excursions to and less scanning of the open arms, indicating that at high dosage the phytopharmacon had anxiogenic properties. These findings confirm and extend the results of a preliminary study (unpublished data) that demonstrated a biphasic dose–response effect of systemic ZC on the behavior of rats in the plus-maze with an anxiolytic-like action at the low dosage, and an anxiogenic-like one with the high dosage.

The anxiolytic profile of ZC revealed in the present experiment showed some parallels, but also some differences with regard to the known anxiolytic profile of benzodiazepines in this model (10,12,29,30). The phytopharmacon was at least as effective as the reference dosage of DZ in elevating the time spent on the open arms as well as increasing open arm end-excursions. However, unlike DZ, the treatment with 0.5 mg/kg ZC failed to increase open arm scanning and did not attenuate risk-assessment. It is possible that these differences of action were due to the increase in locomotor activity observed for ZC but not for DZ, which could have interfered with both risk-assessment and open-arm scanning.

The pharmacological mechanisms that might account for the anxiolytic as well as anxiogenic effects of ZC have yet to be determined. While in general blockade of serotonin, including blockade of 5-HT_3 receptors, has been shown to lead to anxiolytic effects using the elevated plus-maze, such effects are not always consistent (13,35) and may even be contradictory (see Ref. 17 for a review). However, the present results obtained for systemically administered ZC support the hypothesis that blockade of the 5-HT_3 receptor can lead to anxiolytic effects. Several active components of ginger, including gingerol and the diterpenoid galanolactone, were found to exert antiserotonergic activity in the guinea pig ileum assay (21,36). Furthermore, the antiserotonergic effect observed for galanolactone was much greater in guinea pig ileum, which has mainly 5-HT_3 receptors, than that in bioassays that are largely devoid of 5-HT_3 receptive sites, such as the rat fundus or rabbit aorta. In addition, the antagonistic effects of the diterpenoid in guinea pig ileum were most prominent in response to a selective 5-HT_3 agonist and in the presence of selective 5-HT_1 and 5-HT_2 antagonists, demonstrating that the anti5-HT action of the ginger constituent is related to antagonism at 5-HT_3 receptors (21). Extracts of *ginkgo biloba*, on the other hand, can act as indirect serotonin antagonists by preventing the aggregation of thrombocytes (16) and in this way can augment the antiserotonergic effects of ginger. Recently, the compound ZC, the combination of ginger and *ginkgo biloba* extracts, was found to exert antiemetic effects in rats, ferrets, and humans (see Ref. 14 for a review), which were comparable to the known antiemetic action of "classical" 5-HT_3 antagonists (15,26,27). Furthermore, the anxiogenic effects observed for ZC suggest that the compound can have additional activity in the higher dose range. It is feasible that with increasing dosage, ZC exerted 5-HT agonistic properties or was active via neurochemical systems other than 5-HT. Moreover, rats treated with the high dosage of ZC showed a general inhibition of exploratory activity possibly related to the known sedative effects of highly dosed ginger and/or to aversive properties of the compound.

Finally, another important aspect should be pointed out. We recently found that the single components of ZC, at equivalent doses of those that were effective in the present study, were not active in the elevated plus-maze (unpublished data). This suggests that the interaction between ginger and *ginkgo biloba* extracts may be important for the anxiolytic effects of the combination preparation. Concerning the kind of drug interaction, it is possible, for example, that the increase in peripheral (3) and/or central blood flow (23) induced by *ginkgo biloba* extracts can facilitate transport of peripherally administered ginger into the central nervous system (CNS) and by this way enhance the antagonistic action of the compound on central 5-HT neurotransmission.

Taken together, the present data provide evidence that ZC has anxiolytic effects in the elevated plus-maze that are comparable to those of DZ, but that at high dosage the phytopharmacon may also have anxiogenic properties. In general, it appears that anxiolytics impair (24,32), whereas anxiogenic

drugs improve mnemonic processes (34). Thus, with regard to the present findings, it remains to be determined whether ZC has dose-dependent disrupting as well as enhancing effects on the performance of learning and memory tasks.

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