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Dissociation Between Anxiolytic and Hypomnestic Effects for Combined Extracts of Zingiber Officinale and Ginkgo Biloba, as Opposed to Diazepam

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HASENÖHRL, R. U., B. TOPIC, C. FRISCH, R. HÄCKER, C. M. MATTERN AND J. P. HUSTON. *Dissociation between anxiolytic and hypomnestic effects for combined extracts of zingiber officinale and ginkgo biloba, as opposed to diazepam.* PHARMACOL BIOCHEM BEHAV **59**(2) 527–535, 1998.—Previous work has shown that Zingicomb® (ZC), a combination preparation of zingiber officinale and ginkgo biloba, exerts anxiolytic-like effects in the elevated plus-maze (EPM), possibly related to 5-HT antagonistic properties of its components. The first experiment of this study was performed to gauge the specificity of the anxiolytic action of ZC with respect to the mixture ratio of the single components in the combination preparation. Two different combinations of zingiber officinale and ginkgo biloba extracts (ratio of components: 1:1 or 1:2.5) were compared with the standard ratio adjusted for ZC (2.5:1). Each combination was administered intragastrically (IG) in five doses (0.01 to 10 mg/kg) before the rats were tested on the EPM. Zingicomb at 1 mg/kg elevated the time spent on the open arms, scanning of the open arms and excursions into the ends of the open arms, whereas the two other combinations (1:1 and 1:2.5) did not influence rats' behavior on the EPM in the entire dose range tested. With regard to the memory-disrupting effects of anxiolytics, particularly of diazepam (DZP), a second experiment was performed to compare the effects of ZC (0.5, 1, 10 mg/kg, IG) and DZP (1 or 5 mg/kg, IP) on the performance of rats in two different learning tasks. Rats were treated with DZP or ZC prior to the learning trial of a one-trial step-through inhibitory avoidance task. Retention testing 24 h later showed impaired retention for rats injected with DZP at 5 mg/kg but not for animals that had received ZC prior to training. In a further experiment, rats were treated once daily with DZP or ZC prior to the training trials in a water maze. Injections of DZP at 5 mg/kg impaired place and cue learning, whereas the treatment with ZC did not influence the navigation performance in the maze. The present results indicate that the anxiolytic-like effects of ZC are specific in that only the mixture ratio of zingiber officinale and ginkgo biloba adjusted for the phytopharmacon was active in the EPM. Furthermore, ZC did not interfere negatively with the performance on an inhibitory avoidance and a water maze task, as opposed to DZP. This finding is interesting with regard to other studies that have revealed a similar dissociation between anxiolytic and memory-disrupting effects for chemically defined 5-HT antagonists, especially for those acting at 5-HT_3 receptors. © 1998 Elsevier Science Inc.

Anxiolytics Amnesia Benzodiazepine Serotonin 5-HT_3 receptor Ginger

IN the search for alternatives to the benzodiazepines (BZD), antagonists at the serotonin 5-HT_3 receptor are currently being considered for their potential use in the treatment of fear and anxiety-related disorders (12,13). 5-HT_3 receptors are concentrated in hippocampal and amygdala regions of the "limbic system" thought to be involved in fear and anxiety (32), and several studies with rodents and monkeys have shown that 5-HT_3 receptor antagonists have BZD-like anxiolytic effects, but, unlike the BZDs, have no amnestic action or may even facilitate learning when administered either systemically or centrally (2,4,10,44).

Besides chemically defined drugs, there are phytogenics

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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 (28,29,47). Ginkgo biloba extracts, in addition to their well known action in increasing blood flow (35), can act as indirect serotonin-antagonists by inhibition of thrombocyte aggregation (24) and by increasing synaptosomal reuptake of 5-HT in the brain (42).

Our previous work has shown that the phytopharmacon Zingicomb, a combination preparation of ginger and ginkgo biloba, exerts antiemetic effects in different animals models of emesis and in clinical trials [see (21) for review], which are comparable to the known antiemetic action of classical 5-HT_3 antagonists (23). Moreover, a recent study provided evidence for anxiolytic-like effects of Zingicomb in the elevated plus-maze test of fear and anxiety (26), which were dose dependent and similar to those observed for diazepam in this paradigm. However, unlike Zingicomb, equivalent doses of ginger or ginkgo biloba were not active in the EPM when given alone, rather than in combination, raising the possibility that a synergistic interaction between the two components could be essential for the anxiolytic-like effects of the combination preparation.

A close relationship between anxiety and memory processes has been pointed out (45). Brain structures like the amygdala are implicated in both aversive conditioning (7) as well as anxiety (15). Furthermore, in addition to anatomical considerations, pharmacological studies have shown that anxiolytics, particularly the BZDs, can impair (11), whereas anxiogenic compounds like amphetamine or β-carboline can improve mnemonic processes (31,46). Given the relationship between anxiety-reducing and memory-disrupting effects of a drug, it was held possible that the phytopharmacon Zingicomb could have adverse side effects on learning and mnemonic processing similar to those observed after BZD injection. However, a dissociation of anxiolytic and hypomnestic effects was predicted on the basis of recent studies, which demonstrated that chemically defined 5-HT_3 antagonists can exert anxiolytic effects without interfering negatively with the performance on learning tasks (18,20,22).

Based on these findings, the objectives of the present study were twofold. With regard to the above-mentioned interaction between ginger and ginkgo biloba that may be crucial for the anxiolytic effects of Zingicomb, two different mixture ratios of ginger and gingko biloba were compared with the ratio adjusted for the phytopharmacon in their effects on exploratory behavior of rats in the elevated plus-maze. Furthermore, with respect to possible adverse side effects of the phytopharmacon on learning and mnemonic processes, a second experiment was performed that compared Zingicomb with diazepam in effects on retention of an inhibitory avoidance task and on the navigation performance in different versions of the Morris water maze. Both tasks in combination with pretrial drug administration have been shown to be sensitive to measure disruptive effects of BZDs on learning (1,8,36,37), and, thus, were chosen to examine possible hypomnestic or otherwise disruptive influences of the two compounds under investigation.

EXPERIMENT 1: TEST FOR ANXIOLYTIC EFFECTS OF DIFFERENT COMBINATIONS OF ZINGIBER OFFICINALE AND GINKGO BILOBA

Method

Animals. A total of 220 male Wistar rats (Janvier, France), weighing 230–320 g were used for the experiment. Rats were housed in groups of six to eight per cage under standard laboratory conditions with food and water continuously available. A 12 L:12 D cycle was imposed with the lights on from 07:00 to 19:00 h. All behavioral testing was done during the rats' daylight period between 10:00 and 17:00 h. Rats were tail-marked and handled daily for 5 min during the last 3 days before the experiment.

Apparatus. The elevated plus-maze (EPM) 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 [see (40,41) for details]. Illumination was provided by a 40 W red bulb suspended 150 cm above the center of the maze. Wide spectrum 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 rats.

Drugs and injection procedure. Standardized extracts of rhizomes of zingiber officinale (ginger CO_2 extracts, containing 23.5% gingerol) and folia ginkgo biloba, comparable to EGb 761 (ginkgo biloba, containing 24% ginkgoflavonglycosides) were supplied by Mattern et Partner (Starnberg, Germany). Two different mixture ratios of zingiber officinale and ginkgo biloba were used [ratio of ginger: ginkgo biloba = 1:1 (ZC-AA) or 1:2.5 (ZC-inverse)] and compared with the ratio of the components adjusted for Zingicomb (ratio of ginger: ginkgo biloba = 2.5:1). The different combinations of zingiber officinale and ginkgo biloba were dissolved in water with the help of ultrasound, diluted to the desired concentrations with water and administered intragastrically (IG) via a gastric tube. Five different doses of each combination were used ranging from 0.01 to 10 mg/kg. The injections were given in a volume of 2.0 ml/kg body weight; the same volume was used for injecting the diluent vehicle (VEH: water).

Behavioral procedure. The animals received injections of Zingicomb (0.01 mg/kg, $n = 10$; 0.1 mg/kg, $n = 19$; 0.5 mg/kg, $n = 19$; 1 mg/kg, $n = 19$; 10 mg/kg, $n = 10$; VEH, $n = 20$), ZC-inverse (0.01, 0.1, 0.5, 1, and 10 mg/kg, $n = 10$ for each dose; VEH, $n = 11$) or ZC-AA (0.01, 0.1, 0.5, and 1 mg/kg, $n = 10$ for each dose; 10 mg/kg, $n = 11$; VEH, $n = 11$). Each rat received an IG injection and was then returned to its home cage. After 60 min it was placed into the center of the plus-maze, facing one of the enclosed arms. The animals were observed for 5 min, during which the number of entries into and time spent in the open and enclosed arms of the EPM were measured. Furthermore, to examine the "anxiolytic profile" of the treatment, frequency, and duration of scanning (protruding the head over the edge of an open arm and fanning with the vibrissae in any direction), risk-assessment (protruding from an enclosed arm with the forepaws and head only) and end-activity (amount of time spent at the end of an open arm) were determined post hoc for the 5-min experimental session for rats injected with the different doses of Zingicomb, ZC-inverse, ZC-AA, or vehicle. Typically, scanning and end-activity are decreased by anxiogenic drugs, while being increased by anxiolytics; risk-assessment is typically decreased by anxiolytic drugs (14,26).

Statistical analysis. The Mann–Whitney *U*-test was used to test for between-group differences (drug vs. vehicle groups) and Bonferroni's correction of the significance level for multiple comparisons was applied [$\alpha^* = \alpha/n$; n = number of tests; see (34) for details]. The level of significance adopted was $p = 0.05$.

Results

The effects of the different doses of Zingicomb, ZC-inverse, and ZC-AA on the time spent in the enclosed arms, open arms, and central arena of the EPM are depicted in Fig. 1A–C. Rats treated with 1 mg/kg Zingicomb showed a significant increase in time spent on the open arms ($U = 94.0$, $p = 0.003$) and a significant decrease in time spent on the enclosed arms ($U = 95.0$, $p = 0.004$); they did not significantly differ from vehicle controls in time spent in the central arena of the EPM ($U = 134.0$, $p = 0.058$; $\alpha^* = 0.01$; Fig. 1A). Furthermore, the treatment with 1 mg/kg Zingicomb did not influence the number of entries into the enclosed arms ($U = 143.5$, $p = 0.094$) but significantly increased the number of entries into the open arms ($U = 89.0$, $p = 0.002$) as well as the number of total arm entries ($U = 97.5$, $p = 0.005$; data not shown); rats treated with 10 mg/kg Zingicomb also showed an increase in time spent on in the open arms, but the respective p-value missed statistical significance ($U = 65.0$, $p = 0.061$; $\alpha^* = 0.01$). The treatment with the lower doses of Zingicomb (0.01 to 0.5 mg/kg) did not significantly influence the behavioral pattern of the animals (p-values > 0.05). Rats that were treated with the different doses of ZC-inverse (Fig. 1B) or ZC-AA (Fig. 1C) did not significantly differ from vehicle-injected controls in the number of entries into and time spent in the open and enclosed arms of the elevated plus-maze (p-values > 0.05). Table 1 depicts frequency (f) and duration (t) of scanning, risk-assessment and end-activity for rats treated with Zingicomb. Rats treated with 1 mg/kg Zingicomb showed significantly more open arm scanning (f: $U = 94.5$, $p = 0.003$; t: $U = 96.0$, $p = 0.004$) and end-excursion (f: $U = 101.0$, $p = 0.005$; t: $U = 103.0$, $p = 0.005$), whereas risk-assessment was not significantly affected (f: $U = 176.5$, $p = 0.351$; t: $U = 134.0$, $p = 0.058$; $\alpha^* = 0.01$). The behavioral pattern of rats intubated with the higher or the lower doses of Zingicomb did not differ from controls (p-values > 0.05). None of the doses of ZC-inverse or ZC-AA significantly influenced frequency or duration of scanning, risk-assessment and end-activity (p-values > 0.05; data not shown).

EXPERIMENT 2: COMPARISON OF ZINGICOMB WITH DIAZEPAM IN EFFECTS ON INHIBITORY AVOIDANCE CONDITIONING AND WATER-MAZE PERFORMANCE

Method

Animals. A total of 207 male Wistar rats (TVA, University of Düsseldorf; Janvier, France), weighing 230–320 g were used for the experiments (avoidance learning: $n = 123$; water maze: $n = 84$). Rats were housed in groups of six to eight per cage under standard laboratory conditions as described in Experiment 1. Rats were tail-marked and handled daily for 5 min during the last 3 days before the experiment.

Apparatus: avoidance learning. The rats were tested on a one-trial step-trough inhibitory avoidance task described in detail elsewhere (6). In short, the testing apparatus was an oblong plastic box (41 × 24 × 25 cm), which had a guillotine door separating a well-lit transparent start compartment and a

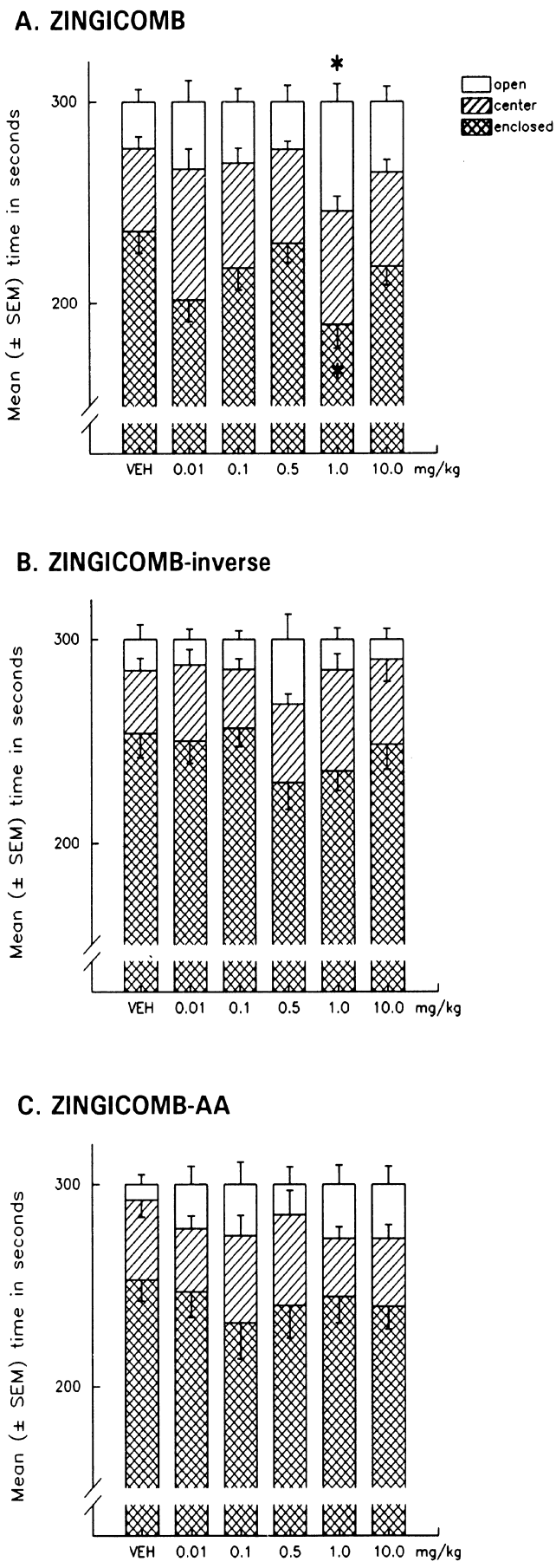

FIG. 1. Mean (±SEM) time in seconds spent in the closed arms, open arms, and central arena of the elevated plus-maze for rats treated with different doses of (A) Zingicomb, (B) Zingicomb-inverse, or (C) Zingicomb-AA. The different combination preparations of zingiber officinale and ginkgo biloba were administered intragastrically in doses ranging from 0.01 to 10 mg/kg 60 min prior to the experimental session. *$p < 0.05$ drug vs. VEH group.

TABLE 1

FREQUENCY AND DURATION OF SCANNING, RISK-ASSESSMENT AND END-ACTIVITY FOR RATS TREATED IG WITH DIFFERENT DOSES OF ZINGICOMB® (ZC) OR WITH THE VEHICLE SOLUTION (VEH) DURING THE 5-MIN TEST PERIOD IN THE ELEVATED PLUS-MAZE

	VEH	ZC 0.01 mg/kg	ZC 0.1 mg/kg	ZC 0.5 mg/kg	ZC 1.0 mg/kg	ZC 10.0 mg/kg
Scanning (f)	3.45 ± 1.08	5.20 ± 1.56	5.00 ± 1.08	4.53 ± 1.48	8.89 ± 1.45*	5.80 ± 1.31
Scanning (t)	10.36 ± 3.43	16.62 ± 7.03	12.63 ± 3.15	13.79 ± 5.10	26.31 ± 4.53*	15.69 ± 4.00
Risk-assessment (f)	10.90 ± 0.80	11.80 ± 0.88	12.47 ± 1.00	12.58 ± 0.74	10.26 ± 0.63	10.50 ± 0.70
Risk-assessment (t)	58.67 ± 6.05	60.68 ± 7.09	70.74 ± 6.74	63.09 ± 7.94	46.08 ± 5.36	50.06 ± 7.20
End-activity (f)	1.10 ± 0.45	2.50 ± 0.78	1.89 ± 0.53	1.58 ± 0.73	3.37 ± 0.71*	2.10 ± 0.78
End-activity (t)	8.45 ± 3.31	18.80 ± 6.11	14.13 ± 4.49	11.38 ± 5.61	24.53 ± 4.98*	14.68 ± 5.07

Values are mean ± SEM; (f) frequency; (t) time in seconds; *$p < 0.05$ drug vs. VEH group.

dimly lit black shock compartment. The shock compartment contained an electrifiable grid floor through which a foot shock could be delivered. The testing device was set up in a sound-attenuating chamber which was lit by a 75 W bulb.

Apparatus: water maze. The water maze was adapted from Morris [see (38) for details]. The animals were tested in a circular pool, 185 cm in diameter and 40 cm in depth. The escape platform used was constructed from 18 cm in diameter black PVC and its height was adjustable. Prior to testing, the maze was filled to a depth of 30 cm with water maintained at 20 ± 1°C. Following each day of testing, the pool was drained and cleaned. The digitized image of the rat's path, tracked by a camera mounted 250 cm above the water surface, was stored and analyzed post hoc by a path analyzer (Chromotrack, San Diego Instruments). The testing environment contained several extramaze cues (stripes on the wall, an arrangement of cages on one side, an uneven structure of the ceiling, the experimenter's desk).

Drugs and injection procedure. Zingicomb (ZC) and diazepam (DZP) were supplied by Mattern et Partner (Starnberg, Germany). The preparation of ZC was as described in Experiment 1. The phytopharmacon was administered IG via a gastric tube in three different doses ranging from 0.5 to 10 mg/kg in a volume of 2.0 ml/kg body weight; the same volume was used for injecting the diluent vehicle (water). DZP (Faustan® solution containing 18.6% EtOH) was dissolved in physiological saline (SAL) and was given intraperitoneally (IP) in two doses (1 or 5 mg/kg) in a volume of 2.0 ml/kg body weight. The doses of DZP used are within the dose range in which the compound had previously been found to disrupt inhibitory avoidance learning (1,8) and to interfere negatively with the navigation performance in a water-maze task (3,36,37).

Behavioral testing procedures: avoidance learning. Rats received injections of either DZP (1 mg/kg, $n = 15$; 5 mg/kg, $n = 13$) or Zingicomb (0.5 mg/kg, $n = 14$; 1 mg/kg, $n = 27$; 10 mg/kg, $n = 14$). Control groups received the respective diluent vehicles: SAL (physiological saline containing two drops of EtOH for DZP, $n = 13$) or VEH (water for ZC, $n = 27$). Sixty minutes after injection (30 min after DZP or SAL) the rats were tested in the step-through task. Each rat had to undergo three habituation (baseline) trials at 1-min intervals. The rat was placed in the start compartment and was allowed to enter the black compartment. Immediately after the rat had entered the dark compartment in the third familiarization trial, the guillotine door was closed and a scrambled foot shock (0.75 mA/1 s) was applied (training). Retention of the step-through response was measured 24 h after shock administration. The animal was placed again in the start compartment and allowed up to 300 s to step-through.

Behavioral testing procedures: water maze. According to their group assignment rats were administered either DZP (1 mg/kg, $n = 12$; 5 mg/kg, $n = 13$) or Zingicomb (0.5 mg/kg, $n = 12$; 1 mg/kg, $n = 12$; 10 mg/kg, $n = 11$). Control groups received the diluent vehicles: SAL for DZP, $n = 12$; VEH for ZC, $n = 12$. Injections were administered once daily 60 min (30 min for DZP or SAL) before testing the animals in the different versions of the water maze using the following protocol [see (25) for details]: on day 1 of maze testing, the rats were habituated to the apparatus by placing each subject in the maze for 90 s with no opportunity to escape. Beginning on day 2, rats were tested in the place version of the task on 4 consecutive days (four trials/day). For each rat, the submerged platform was fixed in the center of one quadrant of the maze below water level. Platform location remained constant for each rat and was counterbalanced within the groups. Rats were placed in the maze facing away from the center of the apparatus from one of four equally spaced points along the perimeter of the pool. Entry points were randomly varied with the criterion that each animal was placed in the maze at each entry point once across every four trials. For all trials, after escaping, rats were allowed to remain on the platform for 30 s. The next trial began 1 min following this 30 s period. A 90-s cutoff was imposed on all trials. If an animal failed to escape during this period, it was placed on the platform by the experimenter for 30 s. On day 6 (spatial probe), the hidden platform was removed, and the rats were placed in the pool for 90 s with no opportunity to escape. Time in platform quadrant (i.e., time spent in the quadrant that had previously contained the escape platform) and "platform crossings" (i.e., number of times the rats swam through the area where the platform had been situated during place learning) were registered. Because both time in platform quadrant as well as platform crossings are not latency measures, they can provide information concerning spatial learning that is largely unconfounded by the motor capacity of the animals. On day 7, animals were tested for four trials in a cued version of the water maze. During these trials, animals were tested as described for the place version with the exception that a platform was placed for each animal 1.5 cm above the surface of the water in the center of the quadrant opposite to its original location. To make the platform more visible, a white PVC cylinder (4 cm in diameter; 3 cm high) was placed on its center. Cue training in the Morris maze permits an analysis of whether animals have sufficient sensory and motor capacities to swim to the platform [see (43) for details]. All behavioral recordings were carried out with the observer unaware of the treatment of the rats.

Statistical analysis. For avoidance learning, the Mann–Whitney *U*-test was used to test for between-group differ-

ences in step-through latency for the retention trial. Fisher's fourfold table test for two categories was used to compare incidence of avoidance (percentage of animals that failed to step-through for the entire 300 s) between groups. For the water maze, individual response curves, defined by the escape latency scores registered on each daily session (total of four trials) in the place version of the water maze task were approximated by an orthogonal polynomial [see (33) for details]. The curve level (a_0) as an index for the average performance in the course of maze testing and the linear trend component (a_1) as an estimate for the acquisition rate were evaluated for between-group differences using the Mann–Whitney *U*-test. Subsequent comparisons of group means across days were also conducted with the Mann–Whitney *U*-test and the same test was used to evaluate differences in rats' performance registered during the spatial probe trial and the cue version of the maze. Whenever multiple comparisons were performed, Bonferroni's correction of the significance level was applied [$\alpha^* = \alpha/n$; n = number of tests; see (34)]. The level of significance adopted was $p = 0.05$.

Results

Avoidance learning. The effects of the different doses of DZP and Zingicomb on retention performance are depicted in Fig. 2. The pretrial injection of DZP disrupted the performance of the step-through task (Fig. 2A). Compared to SAL-injected rats, rats in the DZP 5 mg/kg group had shorter step-through latencies ($U = 48.0$, $p = 0.029$) and none of the animals of this group remained in the start compartment for the entire 300 s (incidence of avoidance: $p = 0.002$; $\alpha^* = 0.025$). The DZP effect was weaker for the DZP 1 mg/kg group (step-through latency: $U = 77.0$, $p = 0.163$; incidence of avoidance: $p = 0.074$), suggesting a dose-dependent effect of DZP in this task. In contrast, pretrial injection of the different doses of ZC did not significantly influence the retention performance (step-through latency: *U*-values ≥ 175.5, *p*-values > 0.05; incidence of avoidance: *p*-values > 0.05; $\alpha^* = 0.017$); rats in the 1 mg/kg Zingicomb group tended to have shorter step-through latencies compared to controls (step-through latency: $U = 287.5$, $p = 0.084$; incidence of avoidance: $p = 0.062$). However, comparisons between training (BL3) and retention latencies indicate that all treatment groups had learned the task (*p*-values < 0.01; Fig. 2B).

Water maze. In the place version of the maze, rats in the DZP 5 mg/kg group showed impaired navigation performance compared to SAL controls as indicated by significantly longer escape latencies across days (a_0: $U = 11.0$, $p = 0.0002$; $\alpha^* = 0.025$), whereas this group did not differ from controls in the rate of acquisition (a_1: $U = 75.0$, $p = 0.435$). A subsequent comparison of the corresponding group means on each test session showed that significant differences in the performance scores of the two latter treatment groups were evident from day 3 onward (*U*-values ≤ 31.0, *p*-values < 0.002; $\alpha^* = 0.025$; Fig. 3A). Rats treated with 1 mg DZP did not differ in navigation performance from controls (a_0: $U = 71.0$, $p = 0.477$; a_1: $U = 69.0$, $p = 0.431$), suggesting a dose-dependent disruptive effect of DZP on place navigation. In contrast, none of the doses of Zingicomb influenced place navigation performance of the animals (a_0: *U*-values ≥ 59.0, *p*-values > 0.05; a_1: *U*-values ≥ 49.0, *p*-values > 0.05; Fig. 3B). On day 6, rats were permitted to swim in the maze for 90 s with the escape platform removed (spatial probe). During this free swim, rats in both DZP groups did not differ in time spent in the quadrant that had previously contained the escape platform (DZP 1 mg/kg: $U = 61.0$; DZP 5 mg/kg: $U = 64.0$; *p*-values > 0.05; Fig. 4A). However, rats treated with 5 mg/kg DZP showed a significantly reduced number of platform crossings compared to controls ($U = 34.0$, $p = 0.008$; $\alpha^* = 0.025$; Fig. 4B). None of the doses of Zingicomb influenced the time spent in the platform quadrant (*U*-values ≥ 63.0, *p*-values > 0.05; Fig. 4A) or the number of platform crossings (*U*-values ≥ 48.0, *p*-values > 0.05; Fig. 4B). On day 7, rats were tested in a cued version of the maze with a visible escape platform fixed in the center of the quadrant situated opposite to the one it was placed into during place learning. Rats treated with 5 mg/kg DZP displayed significantly longer escape latencies than controls ($U = 17.0$, $p = 0.0005$; $\alpha^* = 0.025$), whereas rats in the DZP 1 mg/kg group did not differ from controls ($U = 66.0$, $p > 0.05$; Fig.

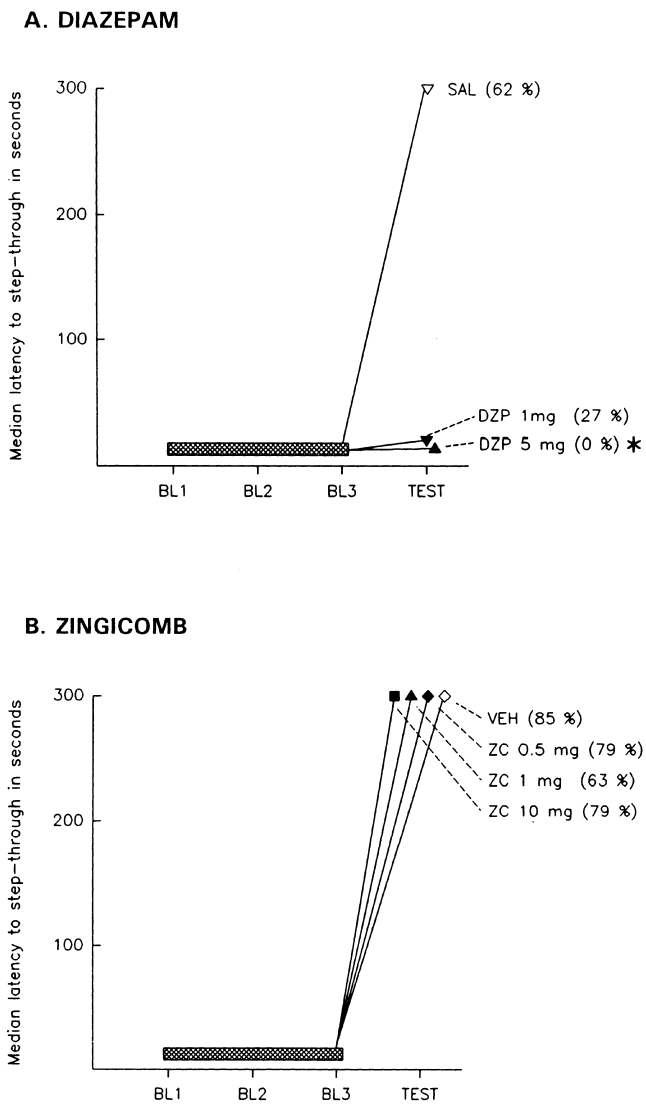

FIG. 2. Median step-through latency for three baseline (BL) trials and the retention test (TEST) for rats that were treated with different doses of (A) diazepam or (B) Zingicomb. All median step-through latencies for the baseline trials fell within the stippled areas. The numbers in parentheses indicate the percentage of rats with step-through latencies longer than 300 s in each group. *$p < 0.05$ drug vs. SAL group.

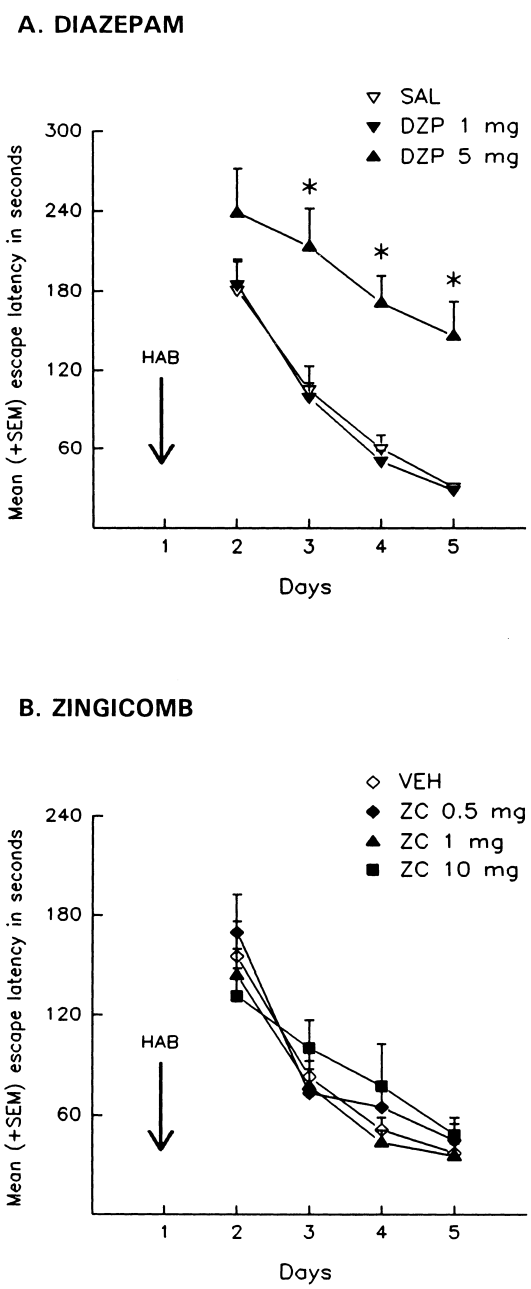

FIG. 3. Navigation performance of rats treated with different doses of (A) diazepam or (B) Zingicomb as measured by the mean (+SEM) time it took the rats to find the hidden platform (place version). *$p <$ 0.05 drug vs. SAL group.

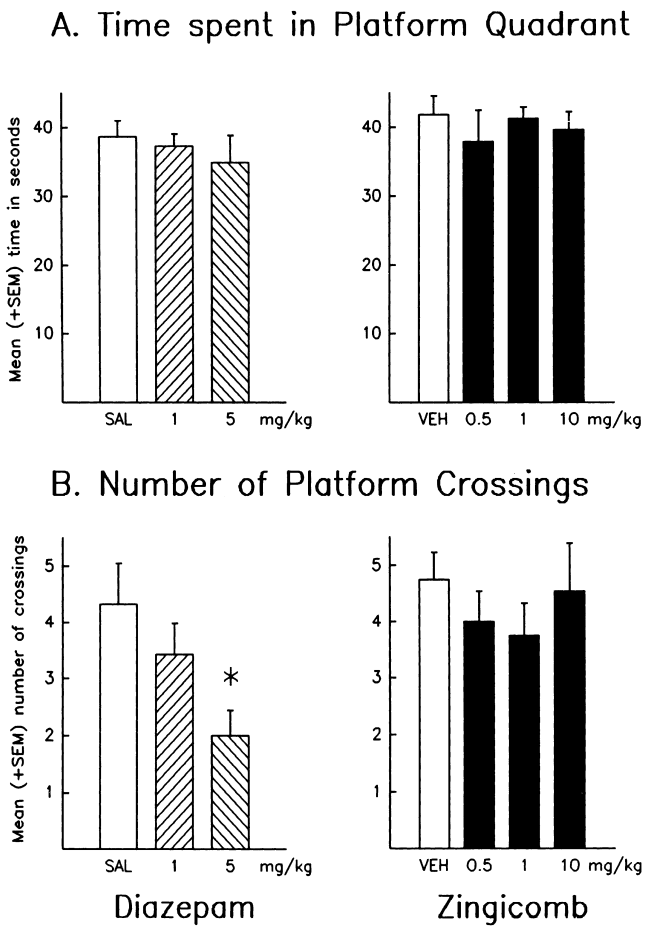

FIG. 4. (A) Mean (+SEM) time in seconds spent in the quadrant of the water maze that had previously contained the escape platform (platform quadrant). (B) Mean (+SEM) number of platform crossings during the spatial probe trial for rats treated with different doses of diazepam (left) or Zingicomb (right). *$p < 0.05$ drug vs. SAL group.

5A). No between-group differences in the navigation performance during the cued trials were evident for rats in the ZC groups (U-values ≥ 56.0, p-values > 0.05; Fig. 5B).

GENERAL DISCUSSION

The results of the first experiment substantiate that Zingicomb can have anxiolytic-like effects in the EPM test of fear and anxiety. In accordance with the results of a previous study (26), the anxiolytic effects of the phytopharmacon were reflected by an inverted U-shaped dose–response function. Systemic injection of Zingicomb at 1 mg/kg increased the number of entries into and time spent on the open arms of the maze. Furthermore, rats in the 1 mg/kg Zingicomb group showed more excursions into the ends of the open arms as well as increased scanning over the edge of the open arms. These effects can be considered to indicate a reduction in fear, and hence, an anxiolytic-like effect of the compound. The lower and the higher dosages of the phytopharmacon were not or even less effective. The treatment with 1 mg/kg Zingicomb did not influence the closed-arm entry scores, which are often used to assess possible treatment effects on general activity. Thus, the increase in time spent on the open arms of the maze evident for Zingicomb-treated rats cannot easily be interpreted in terms of a change in locomotor activity [see (16) for discussion]. We previously found that the single components of Zingicomb, at equivalent doses of those that were effective in the present study, were not active in the EPM (unpublished data). Furthermore, the present results indicate that, unlike Zingicomb, the two other mixture ratios of ginger and ginkgo biloba under investigation, namely Zingicomb-inverse and Zingicomb-AA, did not influence the exploratory behavior of

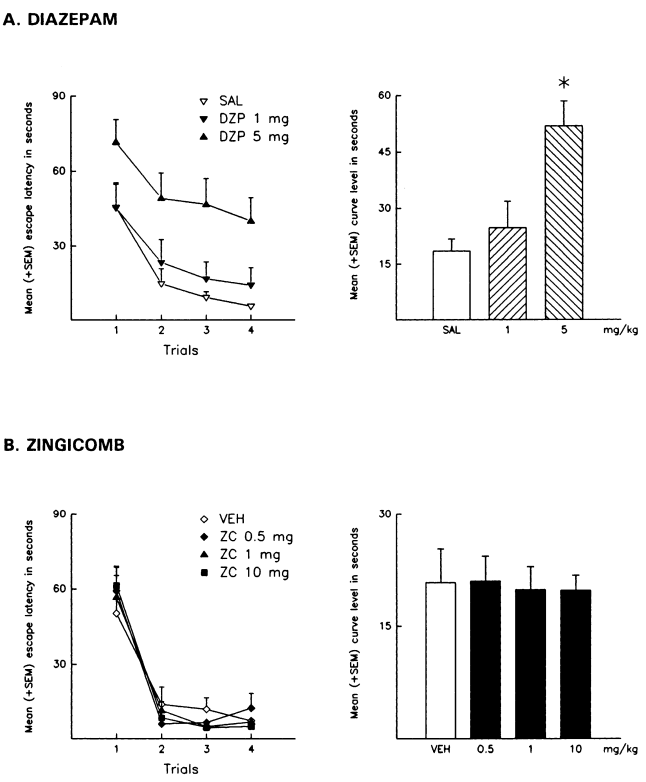

FIG. 5. Maze performance of rats treated with different doses of (A) diazepam or (B) Zingicomb during cue testing as measured by the mean (+SEM) time it took the rats to escape on a visible platform on single trials (left) and during the whole session (right). *$p < 0.05$ drug vs. SAL group.

the animals in the plus-maze. These findings suggest, for one, that the anxiolytic-like effects of Zingicomb may result from a synergistic interaction between ginger and ginkgo biloba extracts and, secondly, provide evidence that the anxiolytic effects of Zingicomb are specific in that only the ratio of the phytogenics adjusted for the phytopharmacon was active. However, the pharmacological mechanisms as well as the kind of drug interaction that might account for the anxiolytic-like effects of Zingicomb have yet to be determined. Concerning the kind of drug interaction, it was proposed that the increase in blood flow induced by ginkgo biloba extracts may facilitate passage of peripherally administered ginger into the brain and by this way could enhance the antagonistic action of the phytopharmacon at central 5-HT_3 receptive sites (26). Furthermore, within the brain ginkgo biloba extracts can facilitate synaptosomal uptake of 5-HT (42) and by this mechanism could sustain the 5-HT_3 antagonistic properties of ginger.

The second series of experiments first of all confirmed that the anxiolytic compound DZP exerts hypomnestic effects when administered prior to acquisition of an inhibitory avoidance and a water maze task. DZP injections administered before training impaired retention of a one-trial step-through avoidance task and the disruptive effect was most prominent in rats that had received the high dosage of the drug (5 mg/kg). The doses of DZP used are within the dose range in which the BZD had repeatedly been reported to disrupt retention performance on one-trial inhibitory avoidance tasks (1) as well as on inhibitory avoidance conditioning in combination with a multi-trial, training-to-criterion procedure (11, 17). Furthermore, several studies have shown that animals treated with DZP prior to training of inhibitory avoidance display amnesia for the task during testing, irrespective of being under DZP or not, suggesting that DZP may not induce state dependency, but, rather may depress acquisition and/or storage of the task [(8,30); but see (39)]. Moreover, congruent with the results of other studies (3,36,37), DZP was found to interfere negatively with the navigation performance in a water maze task. Rats treated with the high dosage of DZP (5 mg/kg) displayed an impairment in learning to navigate the maze when they were tested in the place and cue version of the task as well as during the spatial probe trial. DZP at 1 mg/kg did not influence the rats' navigation performance in the different versions of the maze, suggesting a dose-dependent disruptive effect of DZP in this task. It is interesting to note that in the course of testing the rats in the place version of the task, animals in the DZP 5 mg/kg group were impaired mostly on the first trial of each daily session (the trial that reflects 24-h retention), but did not significantly differ in acquisition rate across daily trials and daily sessions, suggesting that DZP impaired the retention rather than the acquisition of the task. However, at the same dosage, DZP markedly disrupted the performance of the animals on the visible platform task, suggesting that the deficits in learning to navigate the maze are related, in part, to DZP-provoked motivational and/or gross sensorimotor impairments. Most important, however, is the finding that under the same experimental conditions the phytopharmacon Zingicomb did not exert any obvious amnestic or otherwise disruptive effects in the two learning tasks employed. Rats treated with 1 mg/kg Zingicomb tended to have shorter step-through latencies, which could reflect, at most, only weak hypomnestic effects of the phytopharmacon, which, however, are marginal in the light of the strong amnesic action of DZP in this task. During place learning in the water maze, rats treated with Zingicomb were capable of swimming in a goal-directed and coordinated manner and quickly escaped onto the visible platform in the cued trials, as opposed to DZP-treated animals, suggesting that the phytopharmacon had no adverse side effects on spatial learning and memory processes or sensorimotor functions.

Several studies have shown that 5-HT_3 antagonists have BZD-like anxiolytic effects [see, e.g., (5)], but, unlike the BZDs, have no hypomnestic action when administered peripherally or centrally (18,22). Thus, our findings with Zingicomb are in close agreement with those obtained with chemically defined 5-HT_3 antagonists, which suggest a dissociation between their anxiolytic and mnemonic effects. Furthermore, recent studies provided evidence that antagonists at the 5-HT_3 receptor such as ondansetron or SEC-579 can facilitate learning and mnemonic processes in rodents (4,27) and monkeys (2,9,19), raising the possibility that Zingicomb could also have memory-promoting effects. The present results do not rule out such a possibility because the tasks and the experimental design were chosen to investigate possible amnestic or otherwise disruptive influences of the phytopharmacon. Therefore, further experiments using different paradigms would be necessary to examine the possibility that Zingicomb could have mnemonic effects and, in this context, the posttrial application of this compound could be a more suitable test for possible hypermnestic effects of the phytopharmacon.

In sum, the present results indicate that the anxiolytic effects of Zingicomb are specific in that only the specific mixture ratio of ginger and ginkgo biloba adjusted for the combination preparation proved to be active in the elevated plus-maze. Furthermore, Zingicomb did not interfere negatively with the

performance on an inhibitory avoidance and a water maze task, as opposed to DZP, suggesting that the anxiolytic-like effects of the phytopharmacon are dissociable from undesirable side effects on memory processes.

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