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Effects of an Extract of Ginkgo Biloba (EGB 761) on "Learned Helplessness" and Other Models of Stress in Rodents

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PORSOLT, R. D., P. MARTIN, A. LENÈGRE, S. FROMAGE AND K. DRIEU. *Effects of an extract of Ginkgo Biloba (EGB 761) on "learned helplessness" and other models of stress in rodents.* PHARMACOL BIOCHEM BEHAV **36**(4) 963–971, 1990.—The effects of repeated oral administration of an extract of Ginkgo Biloba (EGB 761) on various behavioral models of stress in rodents were investigated. The models in rats included "learned helplessness," shock-suppressed licking (Vogel conflict test) and forced swimming-induced immobility ("behavioral despair"). The models in mice included shock-suppressed exploration (four plates test), spontaneous exploration (staircase test) and food consumption in a novel situation (emotional hypophagia). Further tests in rats examined the effects of EGB 761 on memory (passive avoidance test) and responsiveness to shock to determine whether the preventive effects observed with EGB 761 in the learned helplessness procedure were due either to drug-induced impairment of memory or to reduced shock sensitivity. In all experiments EGB 761 was administered over 5 days at daily doses of 50 and 100 mg/kg PO. In some experiments (Vogel test, four plates test, staircase test, emotional hypophagia) the effects of acute administration were also investigated. The results showed that repeated administration of EGB 761 (50 and 100 mg/kg/day) before exposure to unavoidable shock (preventive treatment) clearly reduced the subsequent avoidance deficits in the learned helplessness procedure but was less effective when first administered after "helplessness" induction (curative treatment). EGB 761 did not affect performance in the passive avoidance task or alter the animals' response to electric shock, suggesting that the effects observed in the learned helplessness procedure were not due to impaired memory or reduced shock sensitivity. Anxiolytic-like activity was also seen in the emotional hypophagia test in mice where repeated administration of EGB 761 increased the amount of food consumed. EGB 761 after acute or repeated administration had no marked effects in the other models tested. The results suggest that EGB 761 reduces the consequences of stress in some experimental situations but that this effect cannot be assimilated to either classical antidepressant or anxiolytic activity.

Extract of Ginkgo Biloba (EGB 761) Animal models of stress Learned helplessness Behavioral despair Conflict Neophobia Memory Shock sensitivity Anxiolytics Antidepressants

THE Ginkgo Biloba is one of the most ancient of all trees and extracts from its leaves and bark have formed part of traditional Chinese medicine over several thousand years (15). More recently, a standardized extract of Ginkgo Biloba leaves (EGB 761), containing 24% flavonol heterosides and 6% terpenes (ginkgolides-bilobalide) (7), has been marketed in Europe (Rökan®, Tanakan®) in particular for its beneficial effects on senility-related cerebral deterioration and functional disturbances following cerebral vascular accidents (16). Clinical publications, including many double-blind crossover studies, have been reviewed recently by Warburton (29) who concluded that EGB 761 was effective in patients with vascular disorder and all types of dementia and exerted some beneficial effects on mood. EGB 761 not only improves the cognitive deficits in geriatric patients but also appears to improve their capacity to cope with the demands of every day living (30). Chronic EGB 761 treatment has been shown to improve vigilance in geriatric patients as measured by EEG criteria (9) and Subhan and Hindmarch (26) have shown that even a single treatment can improve short-term memory in normal volunteers.

In addition to its marked cardiovascular effects (2), EGB 761 has been shown to prolong survival time in mice exposed to hypobaric hypoxia (6) and to decrease the edema induced by experimental embolism with normalisation of brain metabolism and cerebral blood flow (11,21). EGB 761 has been found to attenuate the effects of cerebral infarction induced by arachidonic

acid in rats (4) and to reduce ischemia-induced edema in gerbils (24). EGB 761, after acute and chronic administration, produces a variety of effects on brain amine levels in rats and mice which depend not only on the route of administration but also on the region studied; in rats acute or chronic administration (50 mg/kg PO) causes increases in DA levels in the corpus striatum and bulb with decreases in 5HIAA in the hippocampus, with more marked effects after chronic administration (17). Receptor binding studies indicate that chronic oral EGB 761 (100 mg/kg/day) over 28 days increases the number of muscarinic receptors in the hippocampus of aged Fischer 344 rats (24 months), with a similar but less marked effect in young rats where the muscarinic receptor density is higher (27). Finally, in vitro studies have suggested that EGB 761 can act as a scavenger of free radicals (18).

In standard psychopharmacological tests EGB 761, after acute administration, does not possess a marked profile of activity apart from signs of sedation at high IP doses in various tests (activity meter, psychogalvanic response, barbiturate potentiation, hole board test) (unpublished Pharmacological Dossier Institut Henri Beaufour, 1981). It does not possess classical antidepressant (reserpine antagonism), anxiolytic (four plates test), anticonvulsant (pentetrazol antagonism) or analgesic activity (phenylbenzoquinone-induced writhing).

Other laboratory findings, however, suggest that repeated oral administration of EGB 761 can reduce the effects of experimentally induced stress. In particular, EGB 761 has been found, when administered chronically before exposing rats to a series of inescapable shocks, to prevent the occurrence of performance deficits (learned helplessness) (12) when the animals were subsequently required to learn to avoid shocks of similar intensity. These effects did not appear to be due either to poor memory for the shock or to reduced shock sensitivity. The aim of the present paper is to describe these experiments and to evaluate the effects of EGB 761 in other behavioral situations where spontaneous or consummatory behavior is inhibited by exposure to different forms of stress.

METHOD

Animals

The subjects were male Wistar rats, weighing between 180 and 240 g, and male NMRI mice, weighing between 19 and 25 g, supplied by the Centre d'Elevage Roger Janvier (CERJ), 53940 Le Genest Saint Isle, France. They were delivered to the laboratory at least three days before the experiments and on arrival were housed in groups of 5 rats and 10 mice in transparent macrolon cages (rats: $41 \times 25.5 \times 14.5$ cm; mice: $25.5 \times 19.5 \times 13.5$ cm) containing wood shavings supplied by CERJ with free access to food (U.A.R. 113) and tap water. They were kept in an ambient temperature of 21 ± 1°C under artificial lighting (12 hours) between 8.00 and 20.00.

Drugs

The following drugs were used: Extract of Ginkgo Biloba (EGB 761) (Institut Henri Beaufour), Batch No. K 923 900 178; buspirone hydrochloride (Bristol-Myers); diazepam (Hoffmann-La Roche); imipramine hydrochloride (Sigma); morphine hydrochloride (Coopération Pharmaceutique Française). Soluble compounds (EGB 761, buspirone, imipramine, morphine) were dissolved in distilled water and diazepam, which is insoluble, was dispersed in an aqueous suspension of acacia gum (5%). All drugs were injected in a volume of 5 ml/kg (rats) and 12.5 ml/kg (mice). Doses are expressed as salt or base where appropriate. All experiments were performed in blind conditions using coded solutions.

Procedure

Learned helplessness in the rat. The learned helplessness procedure was similar to that described by Martin *et al.* (14).

Inescapable shock pretreatment. Electric footshocks were delivered in $20 \times 10 \times 10$ cm chambers with Plexiglas walls and cover. The floors were stainless steel grids (1.5 cm mesh). A constant-current shocker was used to deliver 60 scrambled randomized inescapable shocks (15 sec duration, 0.8 mA intensity, every minute $\pm$ 15 sec) to the grid floor. Control rats were placed for 1 hour in identical chambers but no shocks were administered. Inescapable shock pretreatment was performed in the morning on Day 1.

Conditioned avoidance training. In order to evaluate interference effects, avoidance training was initiated 48 hours (Day 3) after inescapable shock pretreatment in automated two-way Ugo Basile shuttle-boxes ($60 \times 21 \times 30$ cm) with Plexiglas walls and a floor consisting of stainless-steel rods spaced 1.0 cm apart. Each shuttle-box was divided into two equal-size chambers by a stainless steel partition with a gate providing access to the adjacent compartment through a 7×7 cm space.

Animals were placed singly in the shuttle-box, were allowed to habituate to the test environment for 5 minutes (for the first session only) and were then subjected to 30 avoidance trials (intertrial intervals: 30 sec). During the first 3 sec of each trial a light signal (used as a CS) was presented allowing the animals to avoid shocks. If no response occurred within this period, a 0.8 mA shock was applied via the grid floor. If no escape response to the shock occurred within 3 sec, the shock and light CS were terminated and an escape failure was recorded. The response (avoidance or escape) required of the rat was to cross the gate into the other compartment of the box. Only 3 sec was permitted for escape since, although escape failure is defined as failure to escape within a 30- to 60-sec period in most procedures used for helplessness assessment, the very first seconds following shock onset seem to be critical for detecting interference effects in animals preexposed to inescapable shocks. Shuttle-box sessions were performed for 3 consecutive days (Days 3, 4 and 5) in the morning and the number of escape failures was recorded.

Effects of EGB 761 given before the stress induction (preventive treatment). To assess the eventual prevention by EGB 761 of the deleterious effects of stress on subsequent avoidance learning different groups of 12 rats were given a single oral administration of EGB 761 per day over 5 days with the last administration being given 90 minutes before the inescapable shock session. The following doses of EGB 761 were investigated: 50 and 100 mg/kg. Diazepam (4 mg/kg/day), administered in the same conditions, was used as a reference compound. The experiment included two control groups, a helpless control which received the same treatment as the drug groups except that, instead of receiving a drug, the animals received oral administrations of distilled water, and a nonhelpless control which received oral administrations of distilled water but was not exposed to inescapable shocks.

Effects of EGB 761 given after the stress induction (curative treatment). To assess the eventual antagonism by EGB 761 of the deleterious effects of prior electric shocks on subsequent avoidance learning, different groups of 12 rats were given repeated administrations of EGB 761 at the following daily doses: 50 and 100 mg/kg, PO. The first administration (50 and 100 mg/kg) was given 6 hours after stress induction on Day 1 and then, at half the quantity per administration, twice a day in the morning (on Days 3, 4 and 5, 90 minutes before the shuttle-box session) and in the afternoon at 16.00 (except the 5th day where 50 and 100 mg/kg

were given only in the morning). Each animal therefore received a total of 8 administrations. Diazepam (4 mg/kg/day, PO), administered in the same conditions, was used as a reference compound. Otherwise, the procedure was identical to that described in the experiment above with helpless and nonhelpless controls.

Passive Avoidance Test in the Rat

To assess the effects of EGB 761 on memory a passive avoidance procedure similar to that described in the mouse by Lenègre *et al.* (10) was employed. On the first day of the passive avoidance task (learning sessions S1) each rat was introduced into the smaller lighted compartment (17 × 17 × 34 cm) of a two-compartment box. When it crossed to the larger darker compartment (49.5 × 31.5 × 34 cm) it received a continuous footshock (0.75 mA) (Apelex, Bagneux, France: ref. 01 1346) until it returned to the lighted compartment. The step-through latency was recorded. Twenty-four hr later, the rat was replaced in the lighted compartment and the step-through latency was recorded with a cut-off at 180 seconds (S2). A longer latency at S2 would indicate that the rat remembered the shock received 24 hr previously. In animals which received an administration of an amnesic agent before S1, a shorter step-through latency at S2 would indicate reduced memory for the shock.

EGB 761 was investigated at the following doses: 50 and 100 mg/kg/day, administered PO twice daily for 4 days at half the indicated dose at each administration and then 90 minutes before S1 on the 5th day. Diazepam (4 mg/kg/day PO), administered in the same conditions, was used as a reference compound. The experiment included a vehicle control group which was treated similarly to the drug groups except that the animals received repeated administrations of distilled water. Twenty animals were studied per group.

Foot-Shock Sensitivity Test in the Rat

To measure the animals' sensitivity to electric shock, a method similar to that described by Charpentier (5) was used. Rats were placed individually into a transparent cage (49.5 × 31 × 34 cm) with a grid floor connected to an electric shock generator (Apelex, Bagneux, France: ref. 01 1346) which transmits a brief electric foot-shock (0.5 sec). Four intensities (0.5, 1, 2 and 4 mA) were investigated. Three shocks were given at each intensity before proceeding to the next higher intensity. The shocks were spaced at 30-second intervals. Response to electric shock was quantified using a scale incorporating three parameters scored 0–3: jump, vocalisation and flight. The total score, obtained for all three parameters at each intensity, was taken as a measure of shock sensitivity.

EGB 761 was investigated at the following doses: 50 and 100 mg/kg/day, administered PO twice daily for 4 days at half the indicated dose at each administration and then 90 minutes before S1 on the 5th day. Diazepam (4 mg/kg/day PO), administered in the same conditions, was used as a reference compound. The experiment included two control groups, a vehicle control group which was treated similarly to the drug groups except that the animals received PO administrations of distilled water and a positive control group which received the same number of administrations of distilled water followed by a single IP injection of morphine (16 mg/kg) 60 minutes before testing. Six animals were studied per group.

Vogel Conflict Test in the Rat

The procedure followed that described by Vogel *et al.* (28). Rats were deprived of water for 48 hours and were then placed individually in a transparent Plexiglas enclosure (31 × 18 × 34 cm) with a floor consisting of stainless steel bars. The back wall of the enclosure was made of nonpainted plywood thereby concealing the observer from the experimental animal. In the centre of the opposite wall, 5 cm above the floor, a metal water spout protruded into the cage and was connected to one pole of a shock generator (Apelex, Bagneux, France: ref. 01 1346). The other pole of the shock generator was connected to the metal grid floor. The rat was left to explore until it found the water spout and then, every time it drank, it received a brief electric shock (1.4 mA; maximum 1 sec) 2 seconds after it started lapping. The test lasted 3 minutes and the number of shocks taken was counted.

EGB 761 was investigated at the following doses: 50 and 100 mg/kg/day, administered PO twice daily for 4 days at half the indicated dose at each administration and then 60 minutes before the test on the 5th day. The effects of acute administrations were also investigated where EGB 761 (50 and 100 mg/kg PO) was administered only once, 60 minutes before the test, to animals which had previously received the same number of administrations of distilled water. Buspirone (32 mg/kg PO), administered acutely in the same conditions, was used as a reference compound. The vehicle control group was treated similarly to the drug groups except that the animals received distilled water at each administration. Ten animals were studied per group.

Behavioral Despair Test in Rats

The procedure followed that described by Porsolt *et al.* (19). The rats were placed individually into glass cylinders (height = 35 cm; diameter = 24 cm) containing 13.5 cm water (25°C) for 15 minutes on the first day of the experiment (D1) and were then put back in the water 5 days later (D5) for a 5-minute test. The duration of immobility during the 5-minute test on D5 was measured. The rats were considered to be immobile when they remained floating in the water making only those movements necessary to stay afloat.

EGB 761 was investigated at the following doses: 50 and 100 mg/kg/day, administered PO twice daily for 4 days at half the indicated dose at each administration and then 60 minutes before the test on the 5th day. The first administration was given immediately after the first exposure to water (D1). Imipramine (32 mg/kg PO), administered repeatedly in the same conditions, was used as a reference compound. The vehicle control group was treated similarly to the drug groups except that the animals received distilled water at each administration. Six animals were studied per group.

Four Plates Test in Mice

The procedure followed that described by Aron *et al.* (1). Mice were individually placed in a white plastic enclosure (25 × 18 × 16 cm) with a floor consisting of four rectangular metal plates (8 × 11 cm). The animal was left to explore freely for 15 seconds and then, for the next 60 seconds, it received a brief electric shock (2 mA; maximum 0.5 seconds) every time it crossed from one plate to another. The number of punished crossings during this period was counted.

EGB 761 was investigated at the following doses: 50 and 100 mg/kg/day, administered PO twice daily for 4 days at half the indicated dose at each administration and then 60 minutes before the test on the 5th day. The effects of acute administrations were also investigated where EGB 761 (50 and 100 mg/kg PO) was administered only once, 60 minutes before the test, to animals which had previously received the same number of administrations

of distilled water. Diazepam (2 mg/kg PO), administered acutely in the same conditions, was used as a reference compound. The vehicle control group was treated similarly to the drug groups except that the animals received distilled water at each administration. Ten animals were studied per group.

Staircase Test in Mice

The procedure followed that described by Stéru *et al.* (25). Mice were placed individually in a white enclosure containing a staircase with 5 steps (height = 2.5 cm; width = 10 cm; depth = 7.5 cm) surrounded by a wall 12.5 cm high. The number of steps climbed and the number of rears were counted during a 3-minute test.

EGB 761 was investigated at the following doses: 50 and 100 mg/kg/day, administered PO twice daily for 4 days at half the indicated dose at each administration and then 60 minutes before the test on the 5th day. The effects of acute administrations were also investigated where EGB 761 (50 and 100 mg/kg PO) was administered only once, 60 minutes before the test, to animals which had previously received the same number of administrations of distilled water. Diazepam (2 mg/kg PO), administered acutely in the same conditions, was used as a reference compound. The vehicle control group was treated similarly to the drug groups except that the animals received distilled water at each administration. Ten animals were studied per group.

Emotional Hypophagia Test in Mice

The procedure followed that described by Soubrié *et al.* (23). Mice were deprived of food 16 hours before the test but had free access to water. They were then placed individually into new macrolon cages (25.5 × 19.5 × 13.5 cm) containing a pellet (approximately 20 g) of nonfamiliar food consisting of powdered UAR 113 small animal diet (70%) compacted into a firm paste with water (30%). The amount of food consumed in 30 minutes was calculated from the difference in the weight of the pellet before and after the test.

EGB 761 was investigated at the following doses: 50 and 100 mg/kg/day, administered PO twice daily for 4 days at half the indicated dose at each administration and then 60 minutes before the test on the 5th day. The effects of acute administrations were also investigated where EGB 761 (50 and 100 mg/kg PO) was administered only once, 60 minutes before the test, to animals which had previously received the same number of administrations of distilled water. Diazepam (2 mg/kg PO), administered acutely in the same conditions, was used as a reference compound. The vehicle control group was treated similarly to the drug groups except that the animals received distilled water at each administration. Ten animals were studied per group.

Statistical Tests

All data were analyzed for overall statistical significance using parametric analysis of variance followed by individual comparisons with the control groups using a two-tailed Dunnett's *t*-test.

RESULTS

Learned Helplessness: Effects of EGB 761 Given Before Stress Induction (Preventive Treatment)

The effects of EGB 761 and diazepam, given repeatedly over 5 days before exposure to inescapable shock (preventive treatment), are shown in Fig. 1. The overall analysis of variance revealed a highly significant difference between the groups in the total

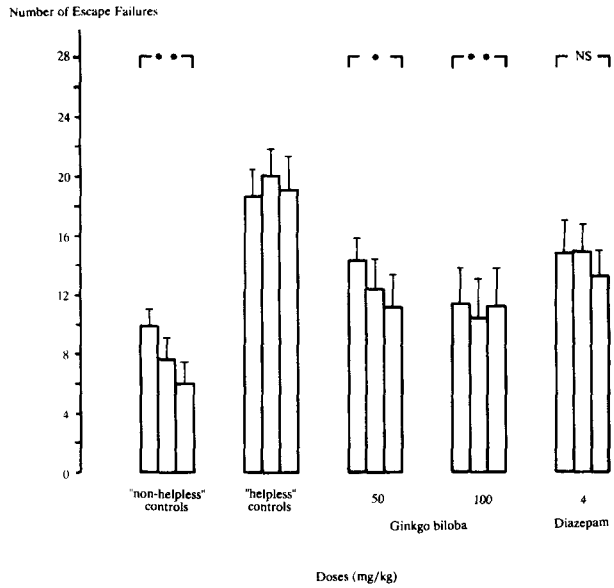

FIG. 1. Learned helplessness in rats—preventive treatment. The effects of repeated PO administration of EGB 761 or diazepam given over 5 days before exposure to prior inescapable shock on the mean (± s.e.m.) number of escape failures during 3 consecutive acquisition sessions (columns 1, 2 and 3 per treatment). The maximum number of escape failures was 30 per session. Doses are expressed as mg/kg/day. The total escape failures for each group (N = 12) were compared with the helpless controls using Dunnett's *t*-test, NS = not significant, $*p<0.05$, $**p<0.01$ (two-tailed).

number of escape failures over the three acquisition trials, $F(4,55) = 5.320$, $p<0.01$.

Individual comparisons showed a highly significant difference between the total number of escape failures in the helpless and nonhelpless control groups, Dunnett's $t(5,55) = 4.408$, $p<0.01$; helpless controls made considerably more escape failures than nonhelpless controls and continued to make a high number of escape failures throughout avoidance acquisition. These results indicate that clear and lasting behavioral deficits (learned helplessness) were induced in control animals by prior exposure to inescapable electric shock.

Preventive treatment with EGB 761 before exposure to inescapable electric shock clearly decreased the total number of escape failures made during acquisition in comparison with helpless controls. This effect was dose-dependent and statistically significant at both 50 mg/kg/day, Dunnett's $t(5,55) = 2.596$, $p<0.05$, and 100 mg/kg/day, Dunnett's $t(5,55) = 3.189$, $p<0.01$. Diazepam (4 mg/kg/day) showed a similar tendency which just fell short of statistical significance, Dunnett's $t(5,55) = 1.960$, $p<0.10$.

Learned Helplessness: Effects of EGB 761 Given After Stress Induction During Avoidance Training (Curative Treatment)

The effects of EGB 761 and diazepam, given during the acquisition of the active avoidance response (curative treatment), are shown in Fig. 2. The analysis of variance revealed a highly significant overall difference between the treatments, $F(4,55) = 6.164$, $p<0.001$.

Individual comparisons showed that there was again a highly significant difference between the helpless and nonhelpless controls, Dunnett's $t(5,55) = 4.393$, $p<0.01$; as in the previous experiment, helpless controls made considerably more escape

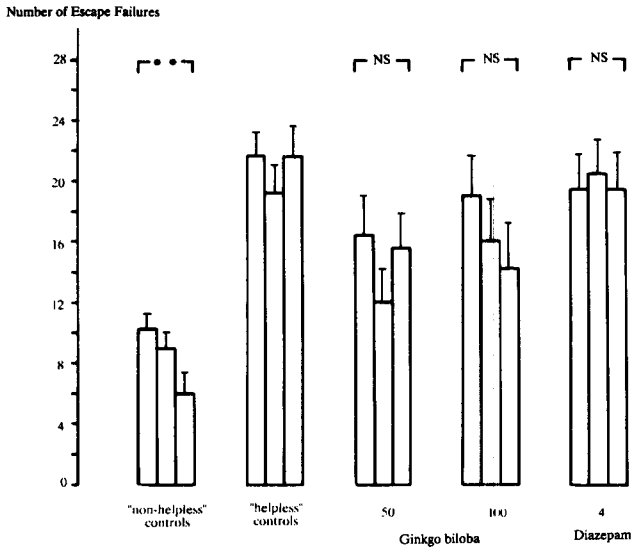

FIG. 2. Learned helplessness in rats—curative treatment. The effects of repeated administration of EGB 761 and diazepam given during avoidance learning on the mean (±s.e.m.) number of escape failures during 3 consecutive acquisition sessions (columns 1, 2 and 3 per treatment). The maximum number of escape failures was 30 per session. Doses are expressed as mg/kg/day. The total escape failures for each group (N = 12) were compared with the helpless controls using Dunnett's *t*-test, NS = not significant, $^{**}p<0.01$ (two-tailed).

failures than nonhelpless controls and continued to do so throughout avoidance training.

EGB 761, when administered repeatedly during the course of avoidance acquisition (curative treatment), had no significant effects on the number of escape failures at either dose tested (50 and 100 mg/kg/day). There was a tendency for rats treated with 50 mg/kg/day EGB 761 to show a decreased total number of escape failures but this effect fell just short of statistical significance, Dunnett's $t(5,55) = 2.151$, $p<0.10$. No such tendency was observed with diazepam (4 mg/kg/day).

Passive Avoidance Task: Effects of EGB 761 on Memory

The effects of EGB 761 and diazepam on performance in the passive avoidance task are shown in Fig. 3. Analysis of variance of the S1 step-through latencies indicates an absence of statistically significant differences between the groups, $F(3,76) = 0.414$, NS. At S2 there was a marked and highly significant increase in the step-through latency as compared with S1 in the nondrug-treated control group [matched $t(18) = 8.210$, $p<0.001$], suggesting that the animals had remembered shock received 24 hours previously. Overall analysis of variance of the S2 step-through latencies revealed a not quite significant treatments effects, $F(3,76) = 2.190$, $p<0.10$.

Inspection of Fig. 3 suggests that EGB 761 was totally without effect on the S2 step-through latencies at either dose tested [Dunnett's $t(4,76) = 0.159$ and 0.300 for 50 and 100 mg/kg/day, respectively, NS], whereas there was a clear tendency for the S2 step-through latency in the diazepam (4 mg/kg/day) group to be lower. Individual comparison with the control group showed this difference to be just short of statistical significance, Dunnett's $t(3,76) = 1.976$, $p<0.10$. These results suggest that diazepam, but not EGB 761, tended to cause memory deficits in this simple learning situation.

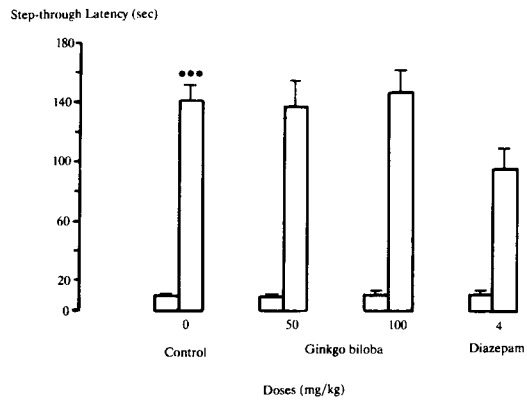

FIG. 3. Passive avoidance test in rats. The effects of EGB 761 and diazepam given PO over 5 days before the first session (S1) on the mean (±s.e.m.) S1 step-through latencies (left columns) and S2 step-through latencies (right columns) of a passive avoidance task (N = 20 per group). The doses are expressed as mg/kg/day. The S1 and S2 latencies in the control group were compared using a paired *t*-test (two-tailed), $^{***}p<0.001$.

Foot-Shock Test: Effects of EGB 761 on Shock Sensitivity

The behavioral response to electric foot-shock after treatment with EGB 761, diazepam and morphine are shown in Fig. 4. To simplify the presentation only the response to 1 mA shock is shown but similar effects at all shock levels were observed with the different drugs tested. Analysis of variance yielded a significant overall treatments effect, $F(4,25) = 4.533$, $p<0.01$.

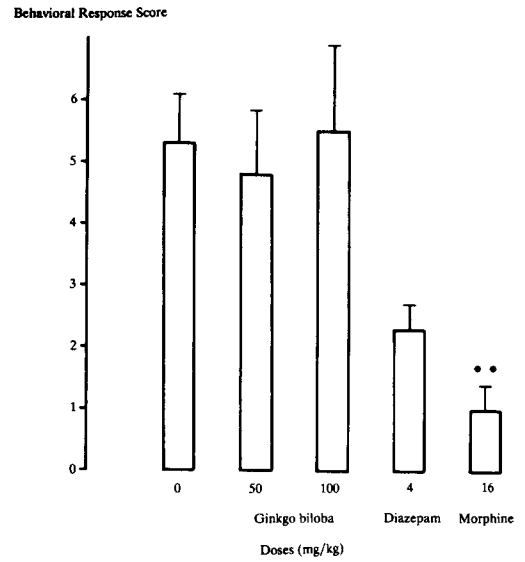

FIG. 4. Shock sensitivity test in rats. The effects of EGB 761, diazepam and morphine on the mean (±s.e.m.) behavioral response to electric shock (1 mA). EGB 761 and diazepam were given repeatedly PO over 5 days and morphine was administered IP once 60 minutes before the test (N = 6 per group). The doses are expressed as mg/kg/day. The behavioral response score for each treatment group was compared with the control scores using Dunnett's *t*-test. $^{**}p<0.01$ (two-tailed).

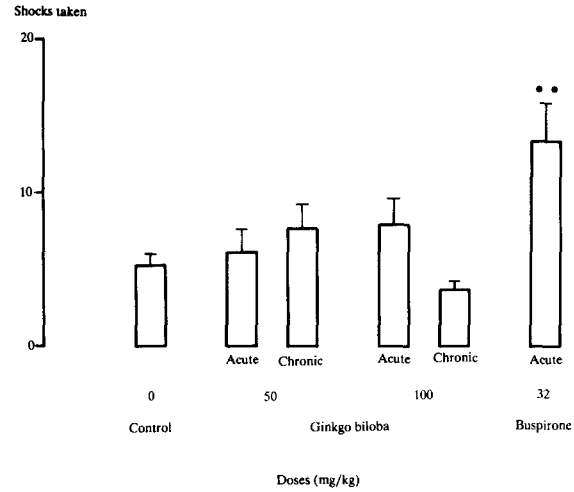

FIG. 5. Vogel conflict test in rats. The effects of EGB 761 and buspirone on the mean (±s.e.m.) number of shocks taken during a 3-minute test. EGB 761 was given PO either acutely or repeatedly over 5 days. Buspirone was given PO once 60 minutes before the test. The graph represents the pooling of two experiments with N = 10 per group. There were therefore 20 animals in the control and buspirone groups. Doses are expressed as mg/kg/day. The score for each treatment group was compared with the control score using Dunnett's *t*-test. $^{**}p<0.01$ (two-tailed).

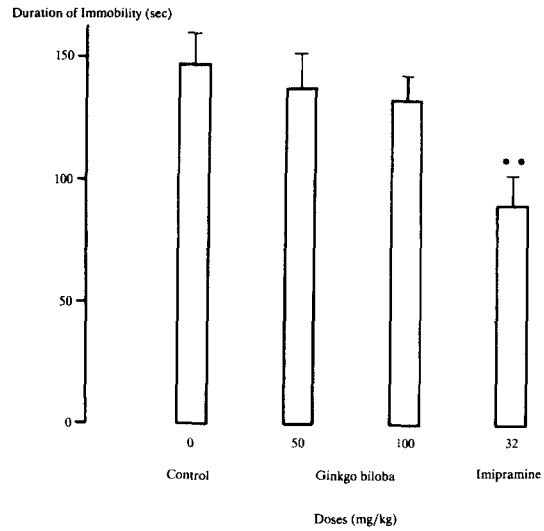

FIG. 6. Behavioral Despair test in rats. The effects of repeated PO administration (5 days) of EGB 761 and imipramine on the mean (±s.e.m.) duration of immobility during a 5-minute test (N = 6 per group). The doses are expressed as mg/kg/day. The immobility score for each treatment group was compared with the control group using Dunnett's *t*-test. $^{**}p<0.01$ (two-tailed).

Inspection of Fig. 4 suggests that EGB 761, at neither dose tested, had any effects on the animals' response to electric foot-shock [Dunnett's $t(5,25)=0.383$ and 0.153 for 50 and 100 mg/kg/day, respectively, NS]. In contrast, an acute injection of morphine (16 mg/kg IP) clearly reduced shock sensitivity, Dunnett's $t(5,25)=3.292$, $p<0.01$, and a similar but not quite significant tendency was observed with 4 mg/kg/day diazepam, Dunnett's $t(5,25)=2.297$, $p<0.10$. These results indicate that EGB 761, unlike morphine or diazepam, did not decrease shock sensitivity in this test situation.

Effects of EGB 761 in the Vogel Conflict Test

The effects of EGB 761 and buspirone in the Vogel conflict test in rats are shown in Fig. 5. The results presented in this figure were obtained by pooling two discrete experiments where the effects of each dose were evaluated separately. There were thus 20 rats in the vehicle and buspirone control groups. Analysis of variance revealed a highly significant overall treatments effect, $F(5,74)=7.572$, $p<0.001$.

Individual comparisons using the Dunnett test revealed that neither acute nor chronic administration of EGB 761 at either dose tested (50 and 100 mg/kg) had any significant effects on the number of shocks taken (50 mg/kg acute: Dunnett's $t=0.390$; 50 mg/kg chronic: Dunnett's $t=1.273$; 100 mg/kg acute: Dunnett's $t=1.377$; 100 mg/kg chronic: Dunnett's $t=0.805$, $df=6,74$, NS). In contrast, a single administration of buspirone (32 mg/kg) caused a marked and highly significant increase in this parameter, Dunnett's $t(6,74)=5.121$, $p<0.01$. These results suggest that EGB 761 was devoid of buspirone-like anxiolytic activity in the Vogel test.

Effects of EGB 761 in the Behavioral Despair Test

The effects of repeated administration of EGB 761 and imipramine in the behavioral despair test in rats are shown in Fig. 6. Analysis of variance yielded a significant overall treatments effect, $F(3,21)=4.402$, $p<0.05$.

Inspection of Fig. 6 suggests that chronic EGB 761 had no effects on the duration of immobility which was confirmed by the results of the Dunnett tests comparing each dose with control (50 mg/kg/day: Dunnett's $t=0.611$; 100 mg/kg/day: Dunnett's $t=0.887$, $df=4,21$, NS). In contrast, chronic imipramine (32 mg/kg/day) clearly decreased the duration of immobility, Dunnett's $t(4,21)=3.379$, $p<0.01$. These results suggest that EGB 761 was devoid of imipramine-like antidepressant activity in the behavioral despair test.

Effects of EGB 761 in the Four Plates Test

The effects of acute and chronic EGB 761 and of acute diazepam in the four plates test in mice are shown in Fig. 7. Analysis of variance yielded a highly significant overall treatments effect, $F(5,54)=9.186$, $p<0.001$.

Inspection of Fig. 7 suggests that neither acute nor chronic administration of either dose of EGB 761 (50 and 100 mg/kg) had any effects on the number of punished crossings. This was confirmed by the results of the Dunnett tests comparing each treatment value with control (50 mg/kg acute: Dunnett's $t=0.0$; 50 mg/kg chronic: Dunnett's $t=0.0$; 100 mg/kg acute: Dunnett's $t=0.70$; 100 mg/kg chronic: Dunnett's $t=0.23$, $df=6,54$, NS). In contrast, acute diazepam (2 mg/kg) clearly increased the number of punished crossings, Dunnett's $t(6,54)=5.399$, $p<0.01$. These results suggest that EGB 761 was devoid of diazepam-like anxiolytic activity in the four plates test.

Effects of EGB 761 in the Staircase Test

The effects of acute and chronic EGB 761 and of acute diazepam on the two parameters measured in the staircase test in mice are shown in Fig. 8. Analysis of variance yielded a highly

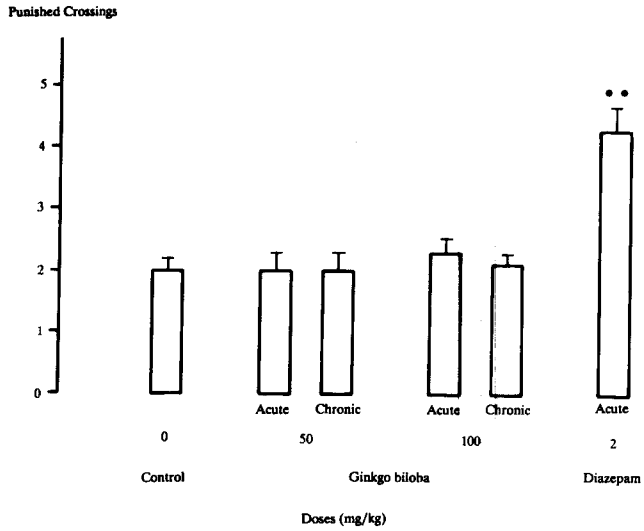

FIG. 7. Four plates test in mice. The effects of acute and repeated (5 days) PO administration of EGB 761 and diazepam on the number of punished crossings during 1-minute test (N = 10 per group). The doses are expressed as mg/kg/day. The score for each treatment group was compared with the control score using Dunnett's *t*-test. $^{**}p<0.01$ (two-tailed).

significant overall treatments effect for the number of steps climbed, $F(5,54)=11.689$, $p<0.001$, and an overall treatments effect just short of statistical significance for the number of rears, $F(5,54)=2.124$, $p<0.10$.

Inspection of Fig. 8 suggests that neither acute nor chronic administration of either dose of EGB 761 (50 and 100 mg/kg) had any effects on the number of steps climbed. This was confirmed by the results of the Dunnett tests comparing each treatment value

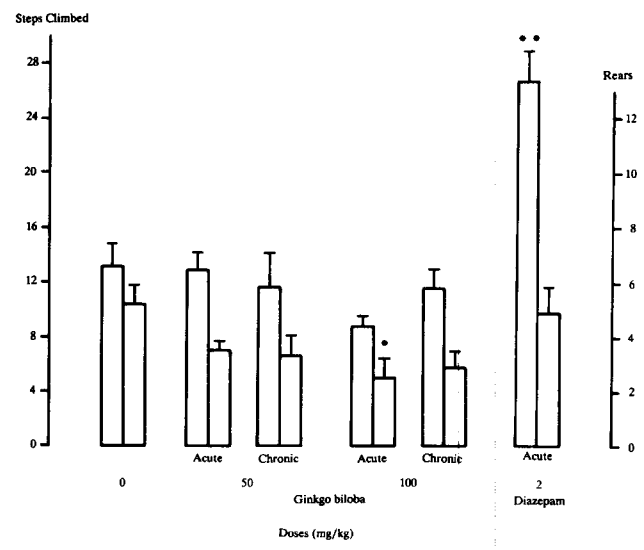

FIG. 8. Staircase test in mice. The effects of acute and repeated (5 days) PO administration of EGB 761 and diazepam on the number of steps climbed (left columns) and the number of rears (right columns) during a 3-minute test (N = 10 per group). The doses are expressed as mg/kg/day. The score for each treatment group was compared with the control group using Dunnett's *t*-test. $^{*}p<0.05$, $^{**}p<0.01$ (two-tailed).

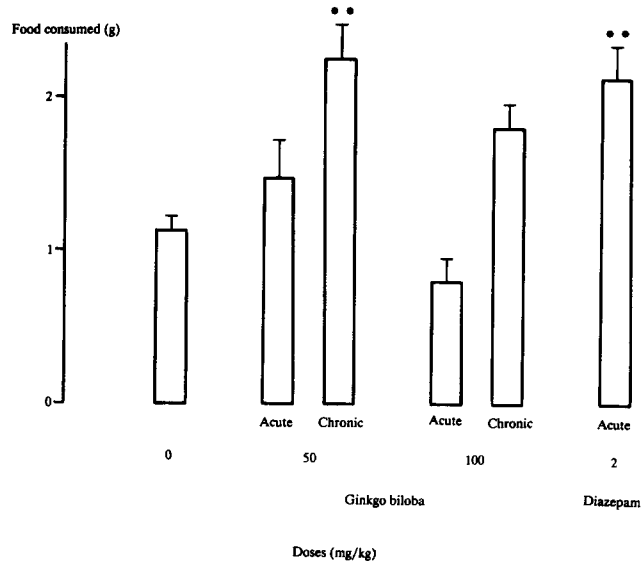

FIG. 9. Emotional hypophagia. The effects of acute and repeated (5 days) PO administration of EGB 761 and diazepam on the quantity of novel food consumed during a 30-minute test (N = 10 per group). The doses are expressed as mg/kg/day. The food consumption score for each treatment group was compared with the control group using Dunnett's *t*-test. $^{**}p<0.01$ (two-tailed).

with control (50 mg/kg acute: Dunnett's $t=0.762$; 50 mg/kg chronic: Dunnett's $t=0.535$; 100 mg/kg acute: Dunnett's $t=1.639$; 100 mg/kg chronic: Dunnett's $t=0.572$, $df=6,54$, NS). In contrast, acute diazepam (2 mg/kg) clearly increased the number of steps climbed, Dunnett's $t(6,54)=5.182$, $p<0.01$.

On the other hand, inspection of Fig. 8 suggests that both acute and chronic administration of 100 mg/kg EGB 761 tended to decrease the number of rears. Individual comparisons with control indicated that the effect observed after acute administration of this dose was statistically significant, Dunnett's $t(6,54)=2.587$, $p<0.05$, whereas the effects observed after chronic administration fell short of statistical significance, Dunnett's $t(6,54)=2.170$, $p<0.10$. In contrast to EGB 761, acute administration of diazepam was totally without effect on this parameter, Dunnett's $t(6,54)=0.283$, NS. These results suggest that EGB 761, after acute or chronic administration, was devoid of diazepam-like anxiolytic activity as indicated by an increase in the number of steps climbed, but after acute administration tended to decrease the number of rears, a sign of sedative activity.

Effects of EGB 761 in the Emotional Hypophagia Test

The effects of acute and chronic EGB 761 and of acute diazepam on the quantity of food consumed by mice in the emotional hypophagia test are shown in Fig. 9. Analysis of variance yielded a highly significant overall treatments effect, $F(5,54)=7.935$, $p<0.001$.

Inspection of Fig. 9 suggests that chronic, but not acute, administration of both doses of EGB 761 increased the quantity of food consumed. This was confirmed by the results of the Dunnett test which showed that acute treatment with EGB 761 was clearly without effect (50 mg/kg acute: Dunnett's $t=1.189$; 100 mg/kg acute: Dunnett's $t=1.153$, $df=6,54$, NS). In contrast, the effect observed after chronic treatment with 50 mg/kg was statistically significant, Dunnett's $t(6,54)=3.957$, $p<0.01$, and that observed

after chronic treatment with 100 mg/kg was just short of statistical significance, Dunnett's $t(6,54) = 2.282$, $p < 0.10$. Acute administration of diazepam (2 mg/kg) clearly increased the quantity of food consumed, Dunnett's $t(6,54) = 3.485$, $p < 0.01$. These results suggest that chronic administration of EGB 761 might have anxiolytic-like effects similar to those observed with diazepam in the emotional hypophagia test.

DISCUSSION

The present experiments have shown that orally administered EGB 761, when given repeatedly before exposing rats to a series of inescapable shocks (preventive treatment), clearly blocks the occurrence of an acquisition deficit (learned helplessness) during the subsequent learning of a conditioned avoidance task. This effect was observed at both doses tested (50 and 100 mg/kg/day) and was more robust than that observed with the standard benzodiazepine diazepam, whose effects in the present experiments were less marked than those we have reported previously (20). Furthermore, in contrast to the clear tendency observed with diazepam, EGB 761 did not induce any impairment in passive avoidance learning or decrease the animals' response to electric shock. These results suggest that the antistress effects of EGB 761, unlike those observed with diazepam, cannot be even partly explained by a decrease in the animals' memory for the shock or to reduced shock sensitivity.

The clear prevention of learned helplessness observed with EGB 761 could appear at first sight to be similar to that observed with benzodiazepines (20,22) and atypical anxiolytics such as buspirone (8) or hydroxyzine (20). The remaining experiments, however, indicated that EGB 761, after acute or chronic treatment, was generally devoid of anxiolytic-like effects in other tests generally used to detect this kind of activity. In particular, EGB 761 was found inactive in the Vogel test, one of the few tests in our hands, which detects not only benzodiazepine activity but also the atypical anxiolytic activity of buspirone. An exception to the above was the finding that chronic EGB 761 increased the quantity of food consumed during the emotional hypophagia test. Such a finding is usually taken to indicate either anxiolytic activity or a stimulation of appetite (23). The latter explanation would appear unlikely, as EGB 761 is not known to affect appetite.

In contrast to its prevention of learned helplessness, EGB 761 did not significantly attenuate helpless behavior when first administered after shock exposure; there was nonetheless a clear tendency to decrease the number of escape failures at 50 mg/kg/day, an effect which just fell short of statistical significance. This slight indication of potential antidepressant activity (13, 14, 22) was not confirmed by the results of the behavioral despair test where, unlike imipramine, EGB 761 had no effects on the duration of immobility. Although the behavioral despair model in rats has been shown to be sensitive to most clinically active antidepressants (3), there are still certain compounds, for example the beta stimulants and the 5-HT uptake inhibitors which are not active in the behavioral despair test but are active against learned helplessness (14). The finding of some behavioral indices of antidepressant activity in rodents would not be inconsistent with Warburton's conclusion that part of the therapeutic efficacy of EGB 761 in geriatric patients is due to beneficial effects on mood (29).

In conclusion, the present experiments suggest that repeated treatment with EGB 761 somehow diminishes the impact of unavoidable stressful stimulation in a manner which cannot be readily assimilated to classical anxiolytic or antidepressant activity. The results obtained in the different models are not sufficiently homogeneous to permit a unitary interpretation but are clear enough to encourage, on the one hand, the search for further situations where such effects can be observed in animals and, on the other hand, a search for the potential relevance of these findings to the clinical use of EGB 761 in man. In this latter respect the clinical observation that EGB 761 not only improves indices of cognitive performance (9,29), but also improves the capacities of elderly patients to cope with the problems of every day life (30) may be of particular relevance.

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