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Interactions of *Ginkgo biloba* Extract (EGb 761), Diazepam and Ethyl β-Carboline-3-Carboxylate on Social Behavior of the Rat

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CHERMAT, R., D. BROCHET, F. V. DEFEUDIS AND K. DRIEU. *Interactions of* Ginkgo biloba *extract (EGb 761), diazepam and ethyl* β*-carboline-3-carboxylate on social behavior of the rat.* PHARMACOL BIOCHEM BEHAV **56**(2) 333–339, 1997.—The social interaction test was used to examine the effects of an extract of *Ginkgo biloba* (EGb 761) and its possible interactions with diazepam and ethyl β-carboline-3-carboxylate (β-CCE). Pairs of naive (unfamiliar) male Wistar AF rats subjected to the same treatment were placed in a novel test arena that was brightly illuminated, and the duration (in s) of social contact was observed over a 10 min period. Single injections of EGb 761 (8–16 mg/kg, IP), given 30 min prior to testing, or repeated oral administration of the extract (48 or 96 mg/kg/day) for 8 days, significantly decreased social contact under conditions that did not influence locomotor activity. Injection of diazepam (1 mg/kg, IP), 30 min before testing, significantly increased social contact. Injection of diazepam to animals that had received repeated oral treatment with EGb 761 (96 mg/kg/day) increased social interaction to an extent greater than observed with diazepam alone. Injection of β-CCE (2–16 mg/kg, IP), 15 min before testing, significantly decreased social contact. When the animals were treated with EGb 761 (48 or 96 mg/kg/day, p.o. for 8 days) and β-CCE (4 mg/kg), both of which decreased social interaction when administered alone, the resulting level of social contact was similar to that of control animals. Interactions with certain sites of central $GABA_A$/benzodiazepine/Cl^- channel receptor complexes could be involved in mediating these effects of EGb 761, diazepam and β-CCE.

Ginkgo biloba extract (EGb 761) | Social interaction test | Anxiolytic activity | Anxiogenic activity | Diazepam

A LEAF EXTRACT of *Ginkgo biloba*, designated EGb 761, is among the most widely employed medicinal plant products in Europe. It is used to treat disturbances in vigilance, short-term memory and other cognitive functions that occur with increasing frequency during ageing and senility, cerebrovascular and peripheral vascular insufficiency, and related neurosensory problems (1,7,9,10,20-22,24,26,36,38). The therapeutic activity of EGb 761 appears to be associated with its actions of increasing glucose uptake and utilization and preserving mitochondrial metabolism and adenosine-5′-triphosphate (ATP) production in various tissues (9,21,23,25,34,35). Several active constituents of EGb 761 appear to be responsible for these actions. The extract contains 24% flavonol glycosides, 6% terpene lactones (ginkgolides, bilobalide), about 7% proanthocyanidins, and certain other constituents (9,28,37). Flavonoids and proanthocyanidins possess free radical-scavenging and enzyme-inhibitor activities (9,27), ginkgolides antagonize the actions of PAF-acether (4) and possess anti-lipoperoxidative activity (6,8,30), and bilobalide may oppose cerebral edema (5) and affect energy metabolism (23,35).

Behavioral studies have revealed that EGb 761 has anxiolytic-like or anti-stress activity in animal models (9). In particular, repeated oral treatment with EGb 761 has been shown to reduce the avoidance deficits induced by unavoidable shock

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in rats (32), to increase food consumption in mice in a novel situation (32), and to exert an anti-stress effect in a discrimination learning task conducted under stressful conditions (33). Other studies have revealed that repeated oral treatment of rats with EGb 761 significantly reduces the hormonal response to an acute surgical stress (29) and prevents cold stress-induced desensitization of hippocampal 5-HT_{1A} receptors in aged animals (2).

Collectively, these findings indicate that the mechanism(s) underlying the anti-stress or anxiolytic-like activity of EGb 761 is distinct from those mediating the actions of classical anxiolytics or antidepressants (32). The present study is aimed at further defining this mechanism. The social interaction test (11,12) was used to examine the possible interactions of EGb 761 with diazepam (an anxiolytic positive control substance in this test that acts as a full agonist at the GABA/benzodiazepine/Cl^- channel complex) and ethyl β-carboline-3-carboxylate (β-CCE; an anxiogenic substance in this test and a partial inverse agonist at the benzodiazepine site of the $GABA_A$ receptor complex)(3,12,14).

METHOD

Subjects

Male Wistar AF rats of homogeneous ancestry (C.E.R.J., Le Genest-St-Isle, France), weighing 180–200 g, were used. The animals were transferred to the laboratory 7 days before commencing experiments and housed in groups of 8 in translucent polycarbonate cages with stainless steel covers (U.A.R. Type 4G) on de-dusted litter (U.A.R., Villemoisson-sur-Orge, France). An ambient temperature of 21 ± 1°C and a light/dark cycle of 10/14 hours (illumination from 0800 to 1800 hr) were maintained throughout the periods of treatment and experimentation. Food (Type 105, U.A.R., France) and tap water were always freely available to the animals. All animals were housed in the same cages from the date of arrival in the laboratory until the end of experimentation, and were tested during the same time period each day (1300 to 1700 hr) to control for the effects that diurnal rhythms might exert on motor activity or on neurotransmitter and hormone release.

Test Substances and Reagents

Extract of *Ginkgo biloba*, EGb 761 (clear maroon-colored powder; lot DM 135/K923) was provided by the Institut Henri Beaufour (Le Plessis Robinson, France). Diazepam (white powder; lot No. 0306019) was purchased from Roche (Neuilly-sur-Seine, France), and ethyl β-carboline-3-carboxylate (β-CCE; lot SC-988A) was purchased from Bioblock Scientific (Illkirch, France). All test substances were dissolved in a solution of gum arabic (0.3%) prepared with distilled water for injectable solutions (lot 1663 A1; Laboratoire Aguettant, Lyon, France), and administered in a volume of 5 ml/kg body weight. EGb 761 was administered either orally or intraperitoneally, as indicated; diazepam and β-CCE were always administered by the intraperitoneal route. Control animals received the drug vehicle.

Experimental Protocol and Treatment of the Animals

The social interaction test (11,17,18), with some minor modifications, was used to examine the anxiolytic/anxiogenic potential of the test substances. This test was conducted using a novel (unfamiliar) test arena (i.e., no previous habituation of the rats), naive (unfamiliar) rats and bright illumination, except for the second phase of experiments conducted with the cross-over design (see below). Under these conditions, social contact between rats is decreased, and anxiolytic drugs are expected to prevent this decline (13).

The light level in the animal housing room was 300 lux. On the day of testing, two rats taken from two different home cages (i.e., pairs of rats that had never been engaged in social contact beforehand) that had received the same treatment were placed in the center of the novel environment (test arena of dimensions 60 × 60 × 45 cm with a solid floor, illuminated by a 100-watt white light bulb situated 50 cm above the floor which provided 600 lux) and the cumulative duration (in sec) of their social contact was scored during a 10-min period. Direct observations were made, and the observer had no prior knowledge of the drug treatment. Zero time was considered to be the moment when the two rats were placed together. To eliminate any bias, such as a cage effect linked to a given treatment, the 8 rats housed in any given cage were given the various treatments in an alternating manner.

A total of 384 rats were used. They were randomly allocated to the various test groups, and the body weights of test partners did not vary more than 10 g. The effects of each dose of test substance and respective control treatments were determined in 8 or 16 rats (4 or 8 pairs). The following interactions were considered to represent positive active contacts: sniffing with or without actual body contact, including that involving the testicles; licking of the congener; crawling of one animal over or under the other; and close pursuit of one animal by the other. Several components of social interaction (e.g., grooming, kicking/boxing, biting and wrestling) were not scored in this study. The test arena was thoroughly cleaned after each test.

The initial series of tests was performed to determine whether or not EGb 761 and diazepam are active in the social interaction test, as employed. A cross-over design was used (4 pairs of rats in all cases). The rats were tested initially 30 min after receiving single injections of EGb 761 or the drug vehicle on Day 1, and then cross-over testing was performed on Day 5; i.e., the interval separating the cross-over was 4 days. The duration of social contact between unfamiliar pairs placed in an unfamiliar environment was monitored for 10 min, and then the same measurements were made under familiar/familiar conditions (i.e., after the animals had been exposed to one another for 4 days).

In further experiments, EGb 761 was tested in single doses of 1–16 mg/kg (IP) and in repeated doses of 24–96 mg/kg/day (PO) for 8 days, diazepam was tested at 1 mg/kg, and β-CCE was tested at 2–16 mg/kg. The first phase of experiments with β-CCE was performed to determine a dose that elicits a submaximal decrease in the duration of social contact, and in the second phase repeated oral treatment with EGb 761 was tested for its possible interaction with this dose of β-CCE. Rats were tested 30 min after receiving EGb 761 (IP) or 60 min after the 8th daily administration of EGb 761 (PO), 30 min after receiving diazepam, or 15 min after receiving β-CCE.

A separate series of experiments was conducted to assess the possible effect of EGb 761 treatment on locomotor activity. EGb 761 was tested in acute doses of 8 and 16 mg/kg (IP) and in repeated doses of 48 and 96 mg/kg (PO), i.e., the doses that were found to be active in the social interaction test. Six groups of eight rats were used, two control (vehicle-treated) groups and four groups representing the active doses of EGb 761; i.e., groups treated with EGb 761 (8 or 16 mg/kg) 30 min before testing; groups treated with EGb 761 (48 or 96 mg/kg/day) for 8 days before testing. In the latter two groups the

final EGb 761 treatment was given 60 min before testing. Locomotor activity was measured in the same experimental arena that was used for social interaction testing, which was subdivided into 9 equal squares, and at the same time of day. Each animal was individually placed in the center of the arena, and the number of squares that were crossed during a 10-min period was counted. A square was considered to have been crossed by the rat when it passed through the pencil line delimiting the square with all four of its paws.

Statistical Analyses

Data are generally expressed as means ± standard errors of the mean (SEM) of the total duration of social contact (in s). For the data shown in Tables 1, 2 and 3 and part of Table 5 (β-CCE, 2 mg/kg), statistical comparisons between treated and control groups were made using Student's *t*-test for non-paired series. For the data of Tables 4 and 6, a Tukey–Kramer multiple comparisons test was used after a two-way ANOVA. For a part of Table 5 (β-CCE, 4–16 mg/kg), Dunnett's multiple comparisons test was used. Homogeneity of variances for Tables 4–6 was shown using Bartlett's test. For Table 7, the results of locomotor testing are represented as means ± SEM of the number of squares crossed. After performing an analysis of variance (one-way ANOVA) and a Bartlett test to verify the homogeneity of variances, a statistical comparison of the mean values was made using Dunnett's test for multiple comparisons.

RESULTS

Intraperitoneal Administration of EGb 761

Single injections of EGb 761 (8 or 16 mg/kg), given 30 min prior to testing, significantly and dose-dependently decreased social contact in the initial phase of cross-over experiments (Table 1). This effect was also evident in the second phase of cross-over experiments in which the level of familiarity could have been increased due to prior exposure of the rats to the testing situation (Table 1).

TABLE 1

EFFECT OF SINGLE INTRAPERITONEAL INJECTIONS OF EGb 761 ON SOCIAL BEHAVIOR IN THE RAT SOCIAL INTERACTION TEST

Testing Condition[1]	Dose of EGb 761 (mg/kg)	Duration of Contact (s)[2]	
		Control[3]	EGb 761
Initial test	1	67.2 ± 6.1	55.0 ± 2.7
Crossover	1	61.0 ± 5.8	53.5 ± 2.6
Initial test	4	59.2 ± 5.0	50.2 ± 5.1
Crossover	4	54.2 ± 3.5	49.0 ± 7.6
Initial test	8	56.5 ± 3.6	34.0 ± 2.7*
Crossover	8	61.5 ± 5.0	26.5 ± 3.1†
Initial test	16	53.0 ± 4.3	15.7 ± 1.6†
Crossover	16	61.0 ± 4.7	18.0 ± 3.8†

Means ± SEM; 8 rats (4 pairs) in all cases; * and † indicate $p < 0.01$ and $p < 0.001$, respectively, for comparisons with corresponding controls (Student's *t*-test for non-paired series.) [1]The rats were tested initially 30 min after receiving single injections of EGb 761 at the doses indicated or the drug vehicle on Day 1, and then cross-over testing was performed on Day 5; [2]duration of social contact between "unfamiliar" pairs placed in a novel environment during a 10-min observation period (and then crossover to "familiar"/"familiar" conditions); [3]controls received the drug vehicle.

Intraperitoneal Administration of Diazepam

Results provided in Table 2 indicate that a single injection of diazepam (1 mg/kg), administered 30 min before commencing the test, significantly increased the duration of social interaction between pairs of unfamiliar rats in a novel environment, as well as in the cross-over phase of this experiment.

Repeated Oral Administration of EGb 761

Administration of EGb 761 (48 or 96 mg/kg/day) for 8 days significantly decreased the duration of social contact between pairs of unfamiliar rats that were placed together in a novel environment, but the dose of 24 mg/kg/day was ineffective (Table 3).

Interaction EGb 761 and Diazepam

A single injection of diazepam (1 mg/kg), given 30 min before commencing the social interaction test, significantly increased social contact (Table 4; see also Table 2). Repeated oral administration of EGb 761 (96 mg/kg for 8 days) had the opposite effect; i.e., it significantly decreased social contact (Table 4; see also Table 3). However, administration of diazepam to animals that had been treated repeatedly with EGb 761 caused an increase in social contact that was more pronounced than that which occurred in vehicle-treated controls (Table 4).

Effect of β-CCE

Injection of β-CCE (2–16 mg/kg) 15 min before commencing the social interaction test caused a significant dose-dependent decrease in the duration of social contact (Table 5).

TABLE 2

EFFECT OF INTRAPERITONEAL ADMINISTRATION OF DIAZEPAM ON SOCIAL BEHAVIOR IN THE RAT SOCIAL INTERACTION TEST

Testing Condition[1]	Duration of Contact (s)[2]	
	Control[3]	Diazepam
Initial test	53.5 ± 5.2	116.0 ± 9.0*
Crossover	50.0 ± 5.0	89.7 ± 2.0*

Means ± SEM; 8 rats (4 pairs) in both cases; * indicates $p < 0.001$ for comparisons with corresponding controls (Student's *t*-test for non-paired series). [1]The rats were tested initially 30 min after receiving single injections of diazepam (1 mg/kg) or the drug vehicle on Day 1, and then the cross-over was performed on Day 5; [2]duration of social contact between "unfamiliar" pairs placed in a novel environment during a 10-min observation period (and then "familiar"/"familiar" conditions with the cross-over); [3]controls received the drug vehicle. In these experiments, the rats were not manipulated as they were in obtaining the data for Table 4, a difference that probably accounts for the more pronounced effect of diazepam (cf. Table 4).

TABLE 3

EFFECT OF REPEATED ORAL ADMINISTRATION[1] OF EGb 761 ON SOCIAL BEHAVIOR IN THE RAT SOCIAL INTERACTION TEST

Dose of EGb 761 (mg/kg)	Duration of Contact (s)[2]
0 (Control)[3]	57.3 ± 4.0
24	56.4 ± 2.4
0 (Control)[3]	69.4 ± 2.8
48	42.9 ± 3.3*
0 (Control)[3]	56.4 ± 3.7
96	24.1 ± 3.1*

Means ± SEM; 16 rats in all cases (i.e., 8 treated pairs and 8 control pairs); * indicates $p < 0.001$ for comparisons with corresponding controls (Student's *t*-test for non-paired series). [1] Rats received EGb 761 at the doses indicated or the drug vehicle (both at 0.5 ml/100 g body weight) for 8 days, the last treatment being given 60 min before commencing behavioral testing on the eighth day; [2] duration of social contact between "unfamiliar" pairs placed in a novel environment during a 10-min observation period; [3] controls received the drug vehicle.

Considering these results, the 4 mg/kg dose of β-CCE, which decreased social contact by at least 30% (sub-maximal dose), was selected for testing its possible interaction with EGb 761 (see below).

Interaction of EGb 761 and β-CCE

Repeated oral administration of EGb 761 for 8 days significantly decreased the duration of social contact by about 24% and 34% at respective doses of 48 and 96 mg/kg (Table 6). Administration of β-CCE (4 mg/kg) 15 min before commencing behavioral testing caused a significant decrease in the duration of social contact (Table 6) that was very similar in magnitude to that observed in the first series of experiments

TABLE 4

INTERACTION OF REPEATED ORAL ADMINISTRATION OF EGb 761 AND SINGLE INTRAPERITONEAL INJECTION OF DIAZEPAM ON SOCIAL BEHAVIOR IN THE RAT SOCIAL INTERACTION TEST

Dose of EGb 761 (mg/kg)	Dose of Diazepam (mg/kg)	Duration of Contact (s)
0[1]	0[2]	56.5 ± 2.4
0[1]	1	69.1 ± 2.1*
96	0[2]	33.6 ± 2.4†
96	1	81.3 ± 3.4†‡

Means ± SEM; 16 rats (8 "unfamiliar" pairs) in all cases; * indicates $p < 0.05$ and † indicates $p < 0.001$ for comparisons with controls; ‡ indicates $p < 0.05$ for the comparison between this value and the value obtained for diazepam alone (Tukey–Kramer multiple comparisons test, after a two-way ANOVA test which showed $F = 55.954$; $p < 0.0001$). Testing was begun 60 min after the 8th daily administration of EGb 761 or the drug vehicle, or 30 min after injection of diazepam. [1,2] Controls received the drug vehicle. The duration of social contact between "unfamiliar" pairs placed in a novel environment was noted during a 10-min observation period. In this series of experiments, the rats were manipulated for 8 days and they appeared to be calmer than those used to obtain the data shown in Table 2, a difference that probably accounts for the less pronounced effect of diazepam (cf. Table 2).

TABLE 5

EFFECTS OF SINGLE INTRAPERITONEAL INJECTIONS OF β-CCE ON THE DURATION OF SOCIAL CONTACT IN THE RAT SOCIAL INTERACTION TEST

Dose of β-CCE (mg/kg)	Duration of Social Contact (s)[1]
0 (Control)[2]	54.4 ± 2.5
2	43.1 ± 3.8*
0 (Control)[2]	55.0 ± 5.4
4	37.2 ± 5.5*
8	30.6 ± 4.2†
16	25.1 ± 2.8†

Means ± SEM; 16 rats (8 pairs) in all cases; * and † indicate $p < 0.05$ and $p < 0.01$, respectively, for comparisons with corresponding control values (for β-CCE at 2 mg/kg, Student's *t*-test for non-paired series was used; for β-CCE at 4–16 mg/kg, Dunnett's multiple comparisons test was used; two-way ANOVA value was $F = 7.896$, $p < 0.001$ for the data concerning the 4–16 mg/kg doses). [1] Duration of social contact between two "unfamiliar" rats placed in a novel environment during a 10-min observation period. [2] In each case, control values pertain to the values shown below them. Rats were tested 15 min after receiving β-CCE or the drug vehicle.

(Table 5) despite the fact that the animals had been subjected to daily manipulations. In animals that had received EGb 761 (48 or 96 mg/kg/day) followed by a single injection of β-CCE, social interaction did not differ significantly from that of controls (Table 6).

EGb 761 and Locomotor Activity

Rats treated with EGb 761 at 8 and 16 mg/kg (IP) or 48 and 96 mg/kg/day (PO) for 8 days showed no statistically

TABLE 6

INTERACTION OF REPEATED ORAL TREATMENTS WITH EGb 761 FOR EIGHT DAYS AND SINGLE INTRAPERITONEAL INJECTIONS OF β-CCE ON SOCIAL BEHAVIOR IN THE RAT SOCIAL INTERACTION TEST

Dose of EGb 761 (mg/kg/day)	Dose of β-CCE (mg/kg)	Duration of Social Contact (s)[1]
0 (Control)[2]	0	58.0 ± 4.1
48	0	44.3 ± 3.9
0	4	39.0 ± 4.4*
48	4	55.5 ± 5.5
0 (Control)[2]	0	56.3 ± 3.9
96	0	37.3 ± 4.8
0	4	35.6 ± 5.7*
96	4	63.0 ± 6.2†

Means ± SEM; 16 rats (8 pairs) in all cases; * indicates $p < 0.05$ for comparisons with corresponding control values; † indicates $p < 0.01$ for the comparison with the corresponding group that received 4 mg/kg β-CCE (Tukey–Kramer multiple comparisons test, after two-way ANOVA tests which showed $F = 3.992$, $p < 0.05$ for the upper 4 values of the table and $F = 6.642$, $p < 0.01$ for the lower 4 values of the table). [1] Duration of social contact between two "unfamiliar" rats placed in a novel environment during a 10-min observation period; [2] control values pertain to the values shown below them. Rats were tested 15 min after receiving β-CCE and 60 min after receiving their eighth daily dose of EGb 761 or the drug vehicle.

TABLE 7

LOCOMOTOR ACTIVITY OF RATS AFTER SINGLE INTRAPERITONEAL INJECTIONS OR REPEATED ORAL ADMINISTRATION OF EGb 761 UNDER CONDITIONS USED FOR THE SOCIAL INTERACTION TEST

Dose of EGb 761	No. of Crossings in 10 min	q^*	$p \leq$
0; Control, IP	48.00 ± 5.91	—	—
8 mg/kg, IP	61.25 ± 5.54	1.618	NS
16 mg/kg, IP	53.25 ± 5.92	0.614	NS
0; Control, PO	55.13 ± 4.89	—	—
48 mg/kg, PO	61.50 ± 6.39	0.738	NS
96 mg/kg, PO	50.13 ± 6.88	0.579	NS

Means ± SEM of the numbers of crossings; 8 animals in all cases. Locomotor testing was performed 30 min after intraperitoneal injection or 60 min after the 8th daily oral administration of EGb 761. No significant differences were noted (Dunnett's multiple comparisons test for which $p < 0.05$ if $q^* > 2.373$).

significant modification of their locomotor activity, as compared with controls (see Table 7). In general, no propensity for sedation was observed in any of the EGb 761-treated rats.

DISCUSSION

The results presented herein, taken together with other recent findings which indicated that EGb 761 exerts anxiolytic-like effects in several other behavioral tests (32,33), strengthen the contention that the mechanism(s) underlying the anti-stress or anxiolytic-like activity of the extract differs from those of conventional anxiolytics or antidepressants (9,32). Thus, unlike classical anxiolytics which increase social contact, and unlike conventional antidepressants which do not influence social contact, in the test employed (12), EGb 761 treatment decreased this parameter. These findings indicate further that although the fundamental action of EGb 761 may be anxiolytic-like, the extract also causes highly significant decreases in social contact when it is administered alone (Tables 1 and 3), effects that are not likely due to a general sedative effect since the locomotor activity of the animals was not decreased (see Table 7). Taking these results together with the observation that EGb 761 acted oppositely to diazepam (which has an anxiolytic profile in this test; see Table 2), this action of the extract may be interpreted as being anxiogenic-like (13). However, some qualification seems necessary. The extract may well have produced a type of false positive result in this regard, since the social interaction test may not differentiate between anxiogenic-like (a negative connotation implying increased anxiety of the organism) and vigilance-enhancing (a positive connotation implying increased awareness of the organism). On this basis, the decreases in social contact elicited by EGb 761 treatment may be more properly termed vigilance-enhancing.

The effect of EGb 761 (administered alone) resembles that of adrenocorticotrophic hormone in that it is not associated with any obvious change in motor activity, rather than that of amphetamine or caffeine whose anxiogenic actions in this test are accompanied by increased motor activity (15,19). On this basis, EGb 761 cannot be characterized as a CNS stimulant. This effect of EGb 761 also resembles that of RO 15-1788 (a benzodiazepine receptor antagonist) which decreases social interaction without modifying motor activity, indicating further that when the extract is administered alone it has an anxiogenic-like rather than a sedative action, the latter of which would be characterized by decreases in both social interaction and motor activity (17). Such an effect of EGb 761 implies an increase in arousal (11).

Treatment of the rats with both diazepam (which increased social contact) and EGb 761 (which decreased social contact) caused a net increase in anxiolytic activity (see Table 4). Although this latter result could, in part, be ascribed to the rather low anxiolytic-like effect of diazepam in the control group of Table 4 (cf. Table 2), it was not entirely surprising in view of previous results which had shown that Ro 15-1788 (which decreases social contact when administered alone) can antagonize both the anxiolytic effects of benzodiazepines and the anxiogenic effect of β-CCE (17). Taken together, such results indicate that the active constituent(s) of EGb 761 exert opposite effects depending upon the prior behavioral state of the animal. If endogenous ligands exist for the benzodiazepine site, EGb 761 could enhance the anxiolytic effect of diazepam by displacing an inhibitory (antagonist-type; anxiogenic) endogenous ligand, or the extract could act via a dual mechanism; i.e., as a partial agonist, which in small doses would exert a benzodiazepine-like effect, but which in large doses would antagonize the effect of an anxiolytic endogenous ligand (17). Alternatively, one might consider that diazepam acted by diminishing EGb 761-induced decreases in social interaction when both agents were administered to the same animal.

Another possibility for explaining the effect of EGb 761 would be that one or more of its constituents inhibited the catabolism of diazepam or its active metabolite, an hypothesis which could be tested in future studies. Speculating further, since the effect of orally administered EGb 761 at 96 mg/kg/day on Day 8 (Table 7) was equivalent to that of acute intraperitoneal administration of 8 mg/kg EGb 761 (Table 1), one might hypothesize that an apparent tolerance had developed to the anxiolytic-like effect of EGb 761 after its chronic administration. However, such results could also be explained simply as being due to the use of different routes of administration (PO vs. IP). Further study with acute administration of EGb 761 would be required to completely eliminate this possibility.

A problem that must be resolved with further testing is the apparent discrepancy that exists between the results reported herein concerning diazepam and previous results obtained with benzodiazepines. The data shown in Tables 2 and 4 indicate that a single intraperitoneal dose of diazepam (1 mg/kg) led to increased social interaction, whereas previous studies have shown that acute administration of chlordiazepoxide caused a dose-related decrease in social interaction (15). Although a firm explanation for this incongruity cannot be provided at this time, it might be noted that species and strain differences exist between these studies and that the dose of chlordiazepoxide used in previous studies (15) decreased both social interaction and locomotor activity (i.e., induced sedation). Also, in the present study the animals were not individually housed prior to testing, indicators of aggressive behavior (e.g., kicking/boxing, biting and wrestling) were not scored, and passive interactions were not evaluated. As these elements contribute to social interactive behaviors (11), such differences in experimental design could have influenced the results obtained.

Like treatment with EGb 761 alone, injection of β-CCE decreased social contact (see Table 6). These results are generally in agreement with those previously reported by the workers who devised the social interaction test. However, in these earlier studies (16,17), administration of β-CCE at doses as low as 1 mg/kg exerted a potent anxiogenic action. In the

present study, higher doses of β-CCE had to be used to produce a quantitatively similar anxiogenic effect (see Table 6). The reason for this discrepancy is likely related to differences in experimental design. In the previous studies (16,17), β-CCE was injected intravenously immediately before behavioral testing because of its rapid metabolism in peripheral tissues, whereas it was administered intraperitoneally 15 min before testing in the present experiments. Also, the rat strain and drug vehicle used in these previous studies differed from those used in the present experiments. The paradigm used in the present study was not modified in assessing the effect of β-CCE because it was deemed necessary to compare all of the results using the same experimental conditions.

Although treatments with either β-CCE or EGb 761 elicited decreases in social interaction in this test, the duration of social interaction of animals that received both treatments did not differ significantly from that of control animals that received only the drug vehicle (see Table 6). Even though EGb 761 decreased social contact when administered alone (anxiogenic-like effect), it exerted an anxiolytic-like effect in opposing (neutralizing) the anxiogenic effect of β-CCE. Thus, it seems likely that the decrease in social interaction produced by EGb 761 treatment occurs via a molecular mechanism different from that which mediates the anxiogenic-like effect of β-CCE. Nevertheless, the molecular mechanism(s) underlying the interactions of EGb 761, diazepam and β-CCE could involve a similar site or distinct sites of action at central $GABA_A$/benzodiazepine/Cl^- channel receptor complexes. Certain small organic acids which are present in EGb 761 (9) or certain flavonoid metabolites that might be formed after oral administration of the extract (31) could interact with specific binding sites of this receptor complex, if such substances penetrate the blood-brain-barrier.

Regardless of the nature of the molecular mechanism, further research in this area could reveal the identity of a constituent of EGb 761 that possesses anxiolytic activity while not inducing the adverse side effects (e.g., sedation, ataxia and amnesia) associated with the use of benzodiazepines. Alternatively, since the extract has anxiogenic-like activity when administered alone, it may be useful in treating those patients who are already taking benzodiazepines or who become inadvertently exposed to β-CCE-like compounds. The finding that EGb 761 treatment decreased social interaction, implying increased arousal (11), is useful in explaining the current clinical use of EGb 761-containing products in treating elderly patients suffering from decreased states of awareness (vigilance), an essential component of cognition.

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