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The Discriminative Stimulus Properties of EGb 761, an Extract of *Ginkgo biloba*

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WINTER, J. C. AND D. TIMINERI. *The discriminative stimulus properties of EGb 761, an extract of* Ginkgo biloba. PHARMACOL BIOCHEM BEHAV **62**(3) 543–547, 1999.—Stimulus control was established in a group of nine rats using a dose of EGb 761 of 10 mg/kg, administered IP, 15 min before training. A two-lever operant task using a fixed-ratio 10 schedule of sweetened milk reinforcement was used. Based upon a criterion for the presence of stimulus control of five consecutive sessions during which 83% or more of all responses were on the appropriate lever, a mean of 24 sessions was required to reach criterion performance. Subsequently, it was observed that EGb 761-induced stimulus control is significantly antagonized by the selective 5-HT_{1A} antagonist WAY-100635, but is unaffected by the 5-HT_2 antagonist pirenperone. Furthermore, EGb 761 generalized to the selective 5-HT_{1A} agonist, 8-hydroxy-dipropylaminotetralin [8-OH-DPAT], and this generalization was blocked by WAY-100635. The present results indicate that EGb 761 is able to induce stimulus control when administered via the intraperitoneal route, and that its stimulus effects are mediated in part by activity at the 5-HT_{1A} receptor. © 1999 Elsevier Science Inc.

EGb-761 Aging Rat Drug-induced stimulus control

THE *Ginkgo biloba* tree has been a part of traditional Chinese medicine for several thousands of years (7). The subject of the present investigation, EGb 761, is a complex yet well-characterized mixture of chemicals obtained from ginkgo leaves via a patented extraction process (7,15). Ginkgo is presently most widely used for the treatment of "cerebral insufficiency," i.e., nonspecific age-related deterioration of mental function (13,14), as well as for degenerative dementias of the Alzheimer and multiinfarct type (10,12,17). Perhaps because of these clinical applications, behavioral studies in intact animals have often focused upon the effects of EGb 761 on learning and memory (19,22,25). In addition, however, several lines of investigation indicate that EGb 761 counters the effects of stress, including the prevention of cold stress-induced desensitization of 5-HT_{1A} receptors (2,20,21). Effects on 5-HT_{1A} receptors are particularly interesting because of their suggested role in learning and memory (3,8,9,27) and the observation that EGb 761 prevents age-associated decreases in the density of this serotonin receptor subtype (11).

The fact that EGb 761 is not a single drug but a complex mixture of chemicals presents formidable barriers to interpretation of results and to the identification of underlying pharmacological mechanisms. In the present investigation, the technique of drug-induced stimulus control, an exceptionally useful technique for the characterization of psychoactive drugs (1,6,23,24), was applied to EGb 761. Specifically, these experiments tested the hypotheses that (a) EGb 761 administered via the intraperitoneal route can establish stimulus control, and (b) the stimulus complex induced by EGb 761 includes a component mediated by serotonergic receptors.

METHOD

Animals

Male Fischer-344 rats were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA) at an age of approximately 6 weeks. They were housed in pairs and allowed free access to water in the home cage. All handling and testing occurred during daytime hours. Standard rat chow was provided immediately following training sessions. Caloric intake was controlled so as to maintain adult body weights of approximately 300 g.

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Apparatus

Two small animal test chambers (Coulbourne Instruments model E 10-10) were used for all experiments. These were housed in larger light-proof, sound-insulated boxes that contained a house light and an exhaust fan. Chambers contained two levers mounted at opposite ends of one wall. Centered between the levers was a dipper that delivered 0.1 ml of sweetened condensed milk diluted 2:1 with tap water. Sessions were managed by a microcomputer using operant control software (Coulbourne Instruments D91-12, version 4.0).

Procedure

Training. After learning to drink from the dipper, rats were trained to press first one and then the other of the two levers. The number of responses for each reinforcement was gradually increased from 1 to 10. During this time, the reinforced lever was alternated on a random basis. All subsequent training and testing sessions used a fixed-ratio 10 (FR10) schedule of reinforcement. Discrimination training was then begun. Fifteen minutes before each 10-min training session, subjects were injected IP with either vehicle or EGb 761. Following the administration of EGb 761, every tenth response on the EGb 761-appropriate lever was reinforced. Similarly, responses on the vehicle-appropriate lever were reinforced on a FR10 schedule following the injection of vehicle. For half of the subjects, the left lever was designated as the drug-appropriate lever. During discrimination training, EGb 761 and saline were alternated on a daily basis. Drug-induced stimulus control was assumed to be present when, in five consecutive

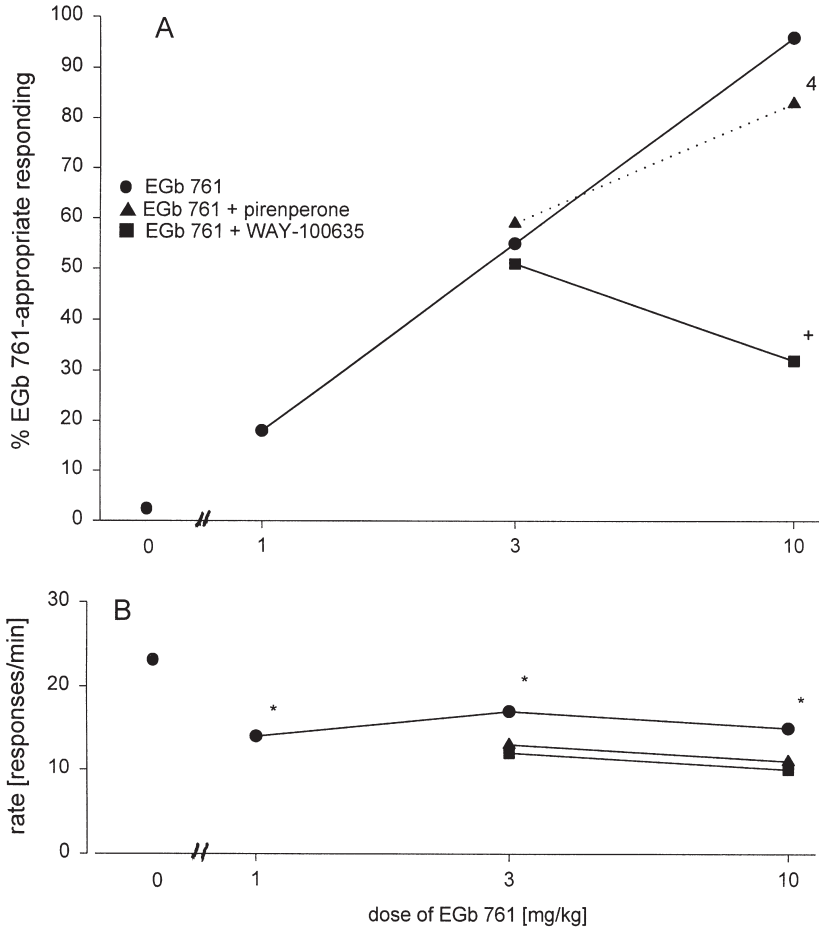

FIG. 1. Dose–response relationship for EGb 761 and interactions with pirenperone and WAY-100635 in rats trained with EGb 761 (10 mg/kg) as a discriminative stimulus. Each point is the mean of one determination in each of nine subjects. Circles represent the effects of EGb 761 alone, squares the effects of EGb 761 following the administration of WAY-100635 (SC; 0.3 mg/kg, 30 min before testing), and triangles the effects of EGb 761 following the administration of pirenperone (IP, 0.16 mg/kg, 60 min before testing). Ordinate: (A) Mean percentage of responses on the EGb 761-appropriate lever; (B) response rate. Abscissa: dose plotted on a log scale. In those instances when not all subjects completed the session an arabic numeral adjacent to a data point in the upper panel indicates the number of animals that completed the session. The data points at zero dose are for the effects of the injection vehicle, a 0.3% solution of gum arabic (acacia). $^{+}$Significantly different from both training conditions. *Significantly different from the vehicle training condition.

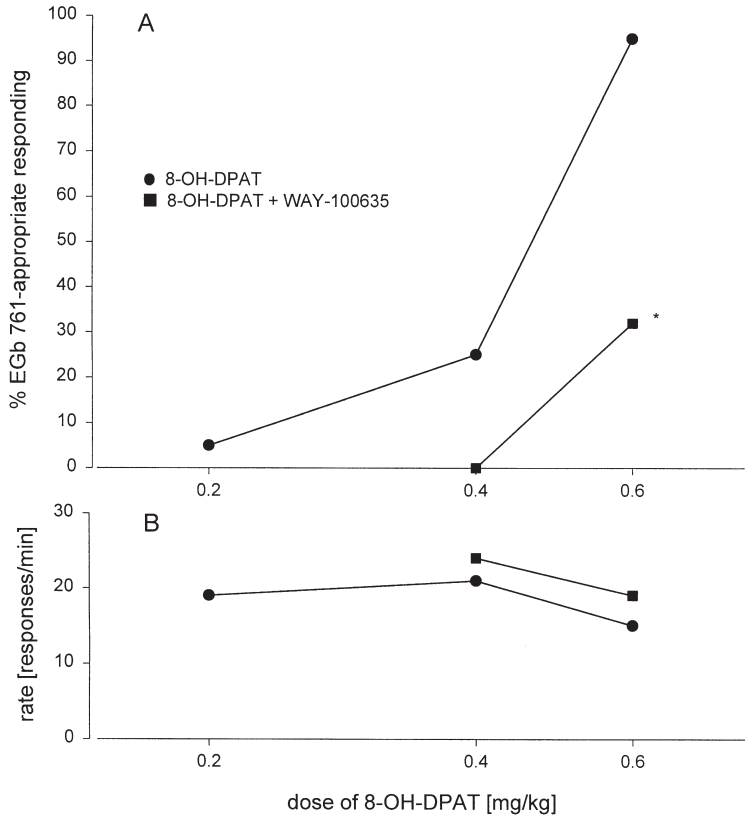

FIG. 2. The effects of 8-OH-DPAT alone (circles) and in combination with WAY-100635 (0.3 mg/kg; squares) in rats trained with EGb 761 (10 mg/kg) as a discriminative stimulus. *Significantly different from 8-OH-DPAT alone. All other details are as in Fig. 1.

sessions, 83 % or more of all responses prior to the delivery of the first reinforcer were on the appropriate lever.

After stimulus control with EGb 761 was well established, substitution tests and antagonism tests were conducted once per week in each animal so long as performance during the remainder of the week did not fall below a criterion level of 83 % correct responding. Subjects were assigned to test groups with the intention of including equal numbers of those trained on the previous day with saline and drug, respectively. During test sessions, no responses were reinforced, and the session was terminated after the emission of 10 responses on either lever. The distribution of responses between the two levers was expressed as the percentage of total responses emitted on the drug-appropriate lever. Response rate was calculated for each session by dividing total number of responses emitted prior to lever selection by elapsed time.

In substitution tests, various doses of 8-OH-DPAT were administered by IP injection 15 min prior to testing. In antagonism tests, WAY-100635 (0.3 mg/kg, SC) or pirenperone (0.16 mg/kg IP) were administered 30 and 60 min, respectively, prior to the initiation of test sessions. Response rates and drug-appropriate responding were recorded as described above.

Statistical Analysis

Data were subjected to one-way repeated measures analysis of variance with subsequent pair-wise multiple comparisons by the method of Student–Newman–Kuels. Differences were considered to be statistically significant if the probability of their having arisen by chance was less than 0.05. An intermediate degree of substitution or antagonism was assumed to be present when test data differed significantly from both training conditions. All analyses were conducted using SigmaStat for Windows™ (Jandel Scientific Software, San Rafael, CA). Criteria for generalization and antagonism were as previously described (28).

Drugs

EGb 761 is the most widely used form of *Ginkgo biloba* in clinical studies, and is standardized in its content of ginkgo flavone glycosides and terpenoids. EGb 761 was obtained from Dr. S. S. Chatterjee, Department of Pharmacology, Dr. Willmar Schwabe GmbH & Co., Karlsruhe, Germany. 8-OH-DPAT HCl was purchased from Research Biochemicals International, Natick, MA. The following drugs were generously provided by the organizations indicated: pirenperone (Janssen Pharmaceutica, Beerse, Belgium), WAY-100635 (Wyeth-Ayerst Research, Princeton, NJ). EGb 761 was dissolved in a 0.3% solution of gum arabic in water (5). All other drugs were dissolved in 0.9% saline solution. All drugs were injected in a volume of 1 ml/kg body weight.

RESULTS

Rats trained with EGb 761 at a dose of 10 mg/kg required a mean of 24 sessions to reach criterion performance. It is seen in Fig. 1 that the training dose was rate suppressant as were

doses of 1 and 3 mg/kg. However, the degree of EGb 761-appropriate responding progressively declined at the lower doses. After stimulus control was well established, approximately 97% of all responses were on the EGb 761-appropriate lever 15 min following the administration of EGb 761. This declined to 46% at 60 min, and to 9% at 120 min (data not shown). Also seen in Fig. 1 are the effects of the 5-$HT_{2A/2C}$ antagonist pirenperone and the 5-HT_{1A} antagonist WAY-100635. Whereas pirenperone was without effect, the effects of the training dose of EGb 761 were reduced to an intermediate level by WAY-100635. In contrast, the rate depressant effects of EGb 761 were further increased by both antagonists and in the case of pirenperone more than half of the subjects failed to complete the test sessions.

In Fig. 2 are shown the results of cross tests with the selective 5-HT_{1A} agonist 8-OH-DPAT in rats trained with EGb 761. At a dose of 0.6 mg/kg, EGb 761 fully generalized to 8-OH-DPAT and this effect was antagonized by WAY-100635.

DISCUSSION

The present results indicate that EGb 761 is efficacious as a discriminative stimulus when administered intraperitoneally and that, using a 15-min pretretment time, its stimulus effects are completely absent after 2 h. Because of the efficacy of EGb 761 as a cognitive enhancer (10,12–14,17), its ability to prevent cold stress-induced desensitization of 5-HT_{1A} receptors (2), and the possible role played by 5-HT_{1A} receptors in memory (3,8,9,27), it was of interest to assess its interactions with the 5-HT_{1A}-selective drugs, 8-OH-DPAT and WAY-100635. Evidence for a 5-HT_{1A}-mediated component in EGb 761-induced stimulus control is provided by (a) antagonism of the EGb 761 cue by WAY-100635 (Fig. 1), and (b) full generalization of *Ginkgo biloba* to 8-OH-DPAT with blockade of that generalization by WAY-100635 (Fig. 2). However, the fact that WAY-100635 did not completely antagonize EGb 761-induced stimulus control suggests the presence of stimulus elements mediated by receptors other than that of the 5-HT_{1A} type. The apparent absence of antagonism by WAY-100635 of a dose of 3 mg/kg EGb 761, despite the fact that the effects of the training dose are diminished to a significant intermediate degree, is puzzling. A plausible explanation is that the element being blocked is present to a greater degree at the training dose than at the intermediate dose of EGb 761, but the data of Fig. 1 do not permit a definitive conclusion to be drawn.

The absence of a significant component mediated by 5-HT_2 receptors is suggested by the fact that pirenperone failed to alter control by EGb 761 despite the use a dose of pirenperone previously shown to completely antagonize the stimulus effects of the 5-HT_2 agonist, DOM (29). Taken together, the effects of 8-OH-DPAT and WAY-100635 in rats trained with EGb 761 seen in the present study are remarkably similar to those recently reported for KA 672 (7-methoxy-6-{3-[4-(2-methoxyphenyl)-1-piperazinyl]propoxy}3,4-dimethyl-2H-1-benzopyran-2-one HCl), a drug under development as a cognitive enhancer and treatment for dementia (18,26).

During the past decade the results of hundreds of investigations of extracts of *Ginkgo biloba* in humans and in animals, in vivo and in vitro, have been published. However, despite this mass of data, the mechanisms by which these extracts might exert their pharmacological effects remains a matter of conjecture. A major factor among the many that contribute to this state of affairs is the fact that tests of learning and memory may be quite time consuming, and hence, are not well suited to the mechanistic assessment of complex mixtures of chemicals such as EGb 761. In contrast, the technique of drug-induced stimulus control is relatively efficient in categorizing the receptor-mediated actions of psychoactive drugs (16), and thus might be expected to contribute substantially to an understanding of the cognitive effects of EGb 761. However, it must be noted that the present investigation differed from most cognitive studies in that the intraperitoneal route of administration was employed. The reason for this choice is that the oral route, as employed in most investigations in intact animals and in virtually all human studies, has only rarely been shown to be efficacious in the establishment of stimulus control in animals (4). In addition, it is possible that the effects of EGb 761 responsible for stimulus control are unrelated to its effects as a cognitive enhancer. Despite these caveats, the present demonstration of EGb 761-induced stimulus control may provide a means to assess previous suggestions as to how EGb 761 might act and to explore novel hypotheses.

In summary, EGb 761, administered via the intraperitoneal route, was shown to induce stimulus control in rats. Furthermore, the generalization of EGb 761 to the selective 5-HT_{1A} agonist 8-OH-DPAT, the full blockade of that generalization by the selective 5-HT_{1A} antagonist WAY-100635, and the partial antagonism of EGb 761 by WAY-100635 are in keeping with a 5-HT_{1A}-mediated component in the stimulus complex induced by of EGb 761.

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