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Ginkgo Biloba Abolishes Aggression in Mice Lacking MAO A

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ABSTRACT

Mice deficient in monoamine oxidase A (MAO A) have increased brain levels of serotonin (5-HT) and norepinephrine and show enhanced aggression. We used MAO A knock-out (KO) mice as a model to study the effect of ginkgo biloba (EGb) on aggression. When EGb was administered to MAO A KO mice, their aggressive behavior in resident–intruder confrontations was reduced to levels seen in wild types. EGb did not affect the locomotive behavior of MAO A KO mice, which suggests that its effects on aggression were not due to sedation. EGb caused a significant 16.9% decrease in [^{3}H]ketanserin binding to 5-HT$_{2A}$ receptors in the frontal cortex of MAO A KO mice but did not change the receptor affinity for [^{3}H]ketanserin. This suggests that the antiaggressive effect of EGb may be mediated by 5-HT$_{2A}$ receptors and that EGb may be developed as a novel antiaggressive agent. Antiox. Redox Signal. 2, 467–471.

INTRODUCTION

GINKGO BILOBA (EGb) is widely used as a herbal remedy to increase short-term memory and cognitive functions in the elderly (Hofferberth, 1994). This is consistent with behavioral studies that have shown that EGb reduces anxiety and increases learning in animals (Petkov *et al.*, 1993; Hasenohrl *et al.*, 1996; Winter, 1998). Most studies have shown that anxiety is enhanced by increasing serotonin (5-HT) activity (Critchley and Handley, 1987) and reduced by decreasing 5-HT activity (Thiebot, 1985; Marsden, 1989; Briley *et al.*, 1990; Critchley *et al.*, 1992). This suggests that EGb may act by reducing 5-HT activity. Similarly, EGb inhibits thromobocyte formation by acting as an indirect 5-HT antagonist (Guinot *et al.*, 1991). EGb is made of many constituents, suggesting that the mechanism that underlies its effects is complex.

Monoamine oxidase (MAO) catalyzes the oxidative deamination of neurotransmitters in the brain and produces hydrogen peroxide (H_2O_2) (Thorpe *et al.*, 1987; Shih *et al.*, 1999a). Two forms of MAO have been defined and they are designated MAO A and B (Johnston, 1968; Knoll and Magyar, 1972). MAO A has higher affinity for 5-HT and norepinephrine (NE) and the inhibitor clorgyline, whereas MAO B has higher affinity for phenylethylamine, benzylamine, and the inhibitor deprenyl. Mice deficient in MAO A have high brain levels of 5-HT and NE and males show high aggression (Cases *et al.*, 1995; Shih *et al.*, 1999a). This is consistent with the impulsive aggression in men

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from a Dutch family with an MAO A deficiency, due to a point mutation of the gene (Brunner *et al.*, 1993a,b).

We used Mao A-deficient mice as a model to study the effect of EGb on aggression. We also determined the effect of EGb on [^{3}H]ketanserin binding to 5-HT$_{2A}$ receptors in the frontal cortex of MAO A knock-out (KO) mice because we have shown that the aggression seen in MAO A KO mice is mediated by 5-HT$_{2A}$ receptors (Shih *et al.*, 1999b).

MATERIALS AND METHODS

Adult male MAO A KO mice aged 1–2 months were used in all studies. The mice were housed individually and were allowed free access to food and water. They were housed under controlled conditions of temperature (20–22°C) and humidity (50–60%) in an air-conditioned unit. Lighting was maintained on a 12-hr light–dark cycle (lights on 0600–1800 hr).

MAO A KO mice were housed individually for at least 1 week in transparent Makrolon cages measuring 29 × 13 × 13 cm. Confrontations were organized in a Latin Square arrangement, and all sessions were at least 2 days apart to prevent cumulative effects of EGb and animal fatigue. Intruder mice were injected with a single dose of 0.1 ml of EGb (Jarrow Formulas™, 6 mg of water-soluble ginkgo biloba 50:1 liquid extract) and were returned to their cage. Controls were injected with 20% alcohol because the EGb solution contains 20% alcohol. After 10 min, an intruder mouse was placed in the cage of the resident mouse. Mice were allowed to interact for 10 min after the first attack. The interactions of the mice were videotaped for later analysis. Observers, blind to the treatment, analyzed the videotape recordings of the encounters for the occurrence of certain stereotyped behaviors. The keys on dedicated keypads were pressed corresponding to the appropriate behavior, and the durations of behaviors were collected and stored using Tufts Event Scoring System software, version 1/30/95 (Princeton Economics, Inc.). Behavior was classified as nonsocial, investigative, defensive, aggressive, and locomotive.

MAO A KO mice were injected intraperitoneally with 0.1 ml of EGb or 20% alcohol as controls. Mice were sacrificed after 10 min; their brain were rapidly removed and the frontal cortex was quickly dissected out on ice. The tissue from two mice was pooled for each assay and was placed in 0.23 *M* sucrose/20 m*M* Tris-HCl, 5 m*M* EDTA, pH 7.4. Homogenates were centrifuged at 6,000 × *g* for 5 min at 4°C. The resulting pellets were resuspended in 20 m*M* Tris-HCl, 10 m*M* EDTA, pH 7.4, using a Polytron homogenizer at speed 6 for 15 sec and centrifuged at 40,000 × *g* for 30 min at 4°C. The resulting pellets were resuspended in 50 m*M* Tris-HCl, 0.5 m*M* EDTA, 0.1% ascorbic acid, and 20 μM pargyline (Sigma), pH 7.4, using a Polytron homogenizer at speed 6 for 15 sec at 10 mg/ml (original wet weight) and kept on ice before the start of binding assays. Then 2-ml aliquots of all homogenates were frozen at −20°C for protein determination using the Pierce BCA kit. 5-HT$_{2A}$ receptor binding (K_D and B_{max}) was measured by [^{3}H]ketanserin (DuPont, specific activity 85.1 Ci/mmol) using saturation assays. Binding (in triplicate) was carried out on an American Rotator V R4140 at 120 rpm in 50 m*M* Tris-HCl, 0.5 m*M* EDTA, 0.1% ascorbic acid, and 20 μM pargyline, pH 7.4, in the dark at room temperature for 60 min. Nonspecific binding was determined with 1 μM mainserin. Binding was terminated by vacuum filtration through glass fiber filters (Whatman GF/B) presoaked for 2 hr in 2% vol/vol polyethylenimine in a one-tenth dilution of 50 m*M* Tris-HCl, 0.5 m*M* EDTA, 0.1% ascorbic acid, and 20 μM pargyline, pH 7.4, at 0°C using a Brandel cell harvester. The filters were washed three times with 5 ml of ice-cold Tris-HCl buffer. Next, 5 ml of Budget-solve was added, and the radioactivity was counted in a Wallac Rackbeta liquid scintillation counter. Counts were corrected for efficiency and decay.

The rating for aggression in the more aggressive mouse of each pair was used to calculate the mean duration of aggression ± SEM. The behavior of the more aggressive mice was statistically compared to the behavior of the least aggressive mice by one-way analysis of variance (one-way ANOVA) followed by post-hoc analysis with the Tukey-Kramer test. Data from saturation experiments were analyzed by Scatchard plots (Scatchard, 1949). From these, measures for K_D and B_{max} (fmol/mg protein)

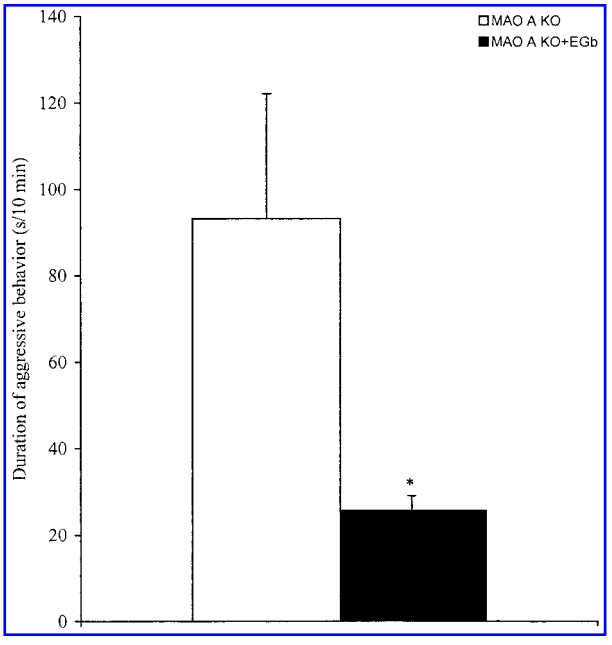

FIG. 1. The effect of EGb on the aggressive behavior of MAO A KO mice in resident–intruder confrontations. The duration of aggressive behavior of the more aggressive mouse from each pair is reported and values are the mean ± SEM ($n = 8$ pairs of mice). Multi-way comparisons (one-way ANOVA and the Tukey-Kramer test) for MAO A KO mice versus MAO A KO mice treated with Egb (*$p < 0.05$).

were calculated. Data from saturation experiments were statistically compared to control values by one-way ANOVA followed by post hoc analysis with Duncan's test. All results were considered significant if $p < 0.05$.

RESULTS

The effect of EGb on aggression was remarkable. When 0.1 ml of EGb was administered to MAO A KO mice, their aggressive behavior in resident-intruder confrontations was reduced significantly (Fig. 1), which is similar to wild types (data not shown). EGb had no effect on nonsocial, investigative, defensive, or locomotive behaviors. Because a decrease in locomotive behavior reflects sedation, these data suggest that EGb did not cause sedation. Twenty percent alcohol had no effect on behavior.

The specific binding of [^{3}H]ketanserin to the frontal cortex of MAO A KO mice was saturable and Scatchard plots were linear ($r > 0.9$), suggesting binding to a single homogenous population of receptors. 5-HT_{2A} receptor binding in the frontal cortex of MAO A KO mice was 80.27 fmol/mg protein, and the K_D for [^{3}H]ketanserin was 0.71 n*M* (Table 1). Values for B_{max} and K_D obtained from the frontal cortex of MAO A KO mice were similar to those previously reported for 5-HT_{2A} receptors (Shih *et al.*, 1999b). EGb decreased [^{3}H]ketanserin binding by 16.9% (66.80

TABLE 1. EFFECT OF EGB ON [^{3}H]KETANSERIN BINDING TO THE FRONTAL CORTEX OF MAO A KO MICE

Genotype	B_{max} *(fmol/mg protein)*	K_D *(nM)*	*Percent decrease in binding*
MAO A KO	80.27 ± 5.19	0.71 ± 0.04	0
MAO A KO + EGb	66.80 ± 5.52[a]	0.65 ± 0.08	16.9
MAO A KO + 20% alcohol	80.41 ± 0.65	0.74 ± 0.01	0

Values are mean ± SEM ($n = 4$). Multi-way comparisons (one-way ANOVA and Duncan's test) for MAO A KO mice versus MAO A KO mice treated with EGb or 20% alcohol.
[a]$p < 0.01$.

fmol/mg protein, $p < 0.01$) but did not change the receptor affinity for [^{3}H]ketanserin. Twenty percent alcohol did not change [^{3}H]ketanserin binding or the receptor affinity of [^{3}H]ketanserin. The protein concentrations of homogenates prepared from the frontal cortex were the same after EGb treatment.

DISCUSSION

This is the first study to show that EGb abolishes aggressive behavior. EGb had no effect on the locomotive behavior of MAO A KO mice, which suggests that EGb did not cause sedation. Previous studies have shown that 0.01 to 10 mg/kg EGb has an anxiolytic effect on rats in the elevated plus-maze test which is reflected as a decrease in the social behavior (Hasenohrl *et al.*, 1996). In our study 0.1 ml of EGb (~250 mg/kg) had no effect on the social behavior of mice in resident–intruder confrontations. This suggests that the anxiolytic effect of EGb is not as marked in resident–intruder confrontations.

MAO A KO mice have increased brain levels of 5-HT and NE and are very aggressive (Cases *et al.*, 1995; Shih *et al.*, 1999a). Using radioligand binding and autoradiography, we have shown that MAO A KO mice have reduced 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_{2C}$ receptors than wild types (Shih *et al.*, 1999b). This may reflect down-regulation by excess 5-HT. EGb decreased [^{3}H]ketanserin binding by 16.9% but did not change the receptor affinity for [^{3}H]ketanserin. This suggests that EGb occupies the 5-HT$_{2A}$ receptor binding site but does not cause a conformational change of the receptor. The protein concentrations of homogenates were the same after EGb treatment. This suggests that changes in [^{3}H]ketanserin binding after EGb treatment are not due to a decrease in total protein levels. The effect of EGb on aggression may be mediated by 5-HT$_{2A}$ receptors. EGb may reduce the aggression of MAO A KO mice by decreasing 5-HT function. Similarly, EGb inhibits thrombocyte formation by blocking 5-HT receptors (Guinot *et al.*, 1991) and may decrease anxiety in animals (Petkov *et al.*, 1993; Hasenohrl *et al.*, 1996; Winter, 1998) by reducing 5-HT activity.

EGb is a mixture of herbal extracts that includes 24% flavonol glycosides and 6% terpene lactones (2.8% bilobalide, 1.2% ginkgolide A, 0.8% ginkgolide B, and 1% ginkgolide C). Flavonoids scavenge free radicals and inhibit many enzymes, including transport ATPases and catechol-*O*-methyl transferase (Middleton, 1984). Ginkgolides inhibit the production of free radicals (Chopra *et al.*, 1993; Corcoran *et al.*, 1993) and bilobalide may protect against cerebral edema (Chatterjee *et al.*, 1986) and hypoxia-induced decreases in ATP (Janssens *et al.*, 1995). EGb may act at α-amino-butyric acid$_A$/benzodiazepine/Cl^- channel receptors (Chermat *et al.*, 1997), and this site may mediate alcohol-induced increases in aggression (Miczek *et al.*, 1993). This suggests that the effect of EGb on aggression may be mediated by 5-HT$_{2A}$ receptors and other mechanisms. Future studies are required to determine the biochemical mechanism that underlies the effect of EGb on aggression.

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ABBREVIATIONS

ANOVA, Analysis of variance; EGb, Ginkgo biloba; H_2O_2, hydrogen peroxide; KO knockout; MAO, monoamine oxidase; NE, norepinephrine; 5-HT, serotonin.

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