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Decreased cerebral 5-HT_{1A} receptors during ageing: reversal by *Ginkgo biloba* extract (EGb 761)

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Abstract—Investigation of [^{3}H]8-hydroxy-2(di-*n*-propylamino)tetralin binding to 5-HT_{1A} receptors in cerebral cortex membranes of Wistar rats showed that the maximal number of binding sites (B_{max}) was reduced significantly (22%) in aged (24-month-old) as compared with young (4-month-old) animals. Chronic treatment with *Ginkgo biloba* extract did not alter binding in young rats but increased binding density significantly (33%) in aged rats. These results confirm previously described age-related 5-hydroxytryptaminergic alterations. Together with data in the literature, they also suggest a restorative effect in aged rats, associated with decreased receptor density resulting from the protective action of *Ginkgo biloba* extract treatment on neuronal membrane.

The present study was undertaken to determine whether some portion of the age-related decrease of 5-HT_1-receptor binding in cerebral cortex is attributable to a decrease in binding to 5-HT_{1A}-subtype receptors. The 5-HT_{1A} receptors in the cerebral cortex of young and aged rats were compared using the specific ligand [^{3}H]8-hydroxy-2(di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT) (Gozlan et al 1983). As *Ginkgo biloba* extract (EGb 761) is widely used against cerebral senescence, the effect of chronic treatment with this product on cerebral cortex 5-HT_{1A} receptors was investigated in young and aged rats. According to a recent report, such chronic treatment increases synaptosomal uptake of 5-hydroxytryptamine (5-HT) in mouse cerebral cortex (Ramassamy et al 1992).

Materials and methods

Drugs. Ginkgo biloba extract (EGb 761) is a well-defined product of green leaves of the Ginkgo tree. Dry leaves are subjected to a 15-step procedure, commencing with an acetone-water extraction under partial vacuum. The final extract is standardized to contain 24% flavonoid glycosides (Ginkgo flavone glycosides) and 6% terpene substances, which are characteristic of *Ginkgo biloba* and have a unique structure (ginkgolides, bilobalide) (Drieu 1986). An injection form was composed of lyophilized EGb 761 (50 mg) and mannitol (100 mg) dissolved in 3 mL distilled water containing 10 mg disodium phosphate. Placebo solution was composed of the excipients only. Solutions were diluted in distilled water.

5-Hydroxytryptamine creatinine sulphate was purchased from Sigma. Pargyline hydrochloride was donated by Abbot (Rungis, France). [^{3}H]8-OH-DPAT (228 Ci $mmol^{-1}$) was obtained from Amersham (Les Ulis, France).

Animals and treatment. Young (4-month) and aged (24-month) male rats (Wistar, CERJ, Le Genest, France) were housed in groups of three and acclimatized for at least one week on a 12-h light–dark cycle. Rats were maintained on U.A.R. AO4 diet and allowed free access to food and water.

Ginkgo biloba extract and the placebo were administered intraperitoneally at a dose of 5 mg kg^{-1} once a day for 21 consecutive days in groups of 18 rats. This dose was chosen according to a previous study on α_2-adrenoceptors (Huguet & Tarrade 1992).

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Membrane preparation. Rats were decapitated 24 h after the last injection of extract or vehicle. Brains were quickly removed and the cerebral cortex was dissected on ice as previously described (Glowinski & Iversen 1966). Tissues were homogenized using an Ultra-Turrax (IKA, Labortechnick, T25) in 10 vol ice-cold 0·25 M sucrose. Nuclear debris was removed by centrifugation (1000 *g*, 10 min, 4°C), and the supernatants were stored at 4°C. Pellets were resuspended in 0·25 M sucrose and recentrifuged (1500 *g*, 10 min, 4°C). The two supernatants were combined, diluted (1 : 3) in 50 mM Tris-HCl buffer (pH 7·6) and centrifuged at 50 000 *g* for 10 min at 4°C (Heraeus 20 RS centrifuge). The resulting pellets were rehomogenized in the same buffer and recentrifuged at 50 000 *g* for 10 min at 4°C. The final pellets were resuspended in ice-cold Tris-HCl 50 mM (pH 7·6) buffer.

Binding studies. Samples (1 mL) of the membrane preparation containing 0·8–1 mg protein were incubated with [^{3}H]8-OH-DPAT at concentrations ranging from 0·8 to 10 nM. Incubation was carried out at 30°C in 50 mM Tris-HCl buffer (pH 7·8; 1 mM $MnCl_2$ and 10 μM pargyline; final volume 2 mL) for 40 min. The reaction was stopped by rapid filtration under vacuum through GF/F filters (Whatman). Filters were immediately washed twice with 5 mL ice-cold buffer and suspended in 7·5 mL premixed liquid scintillation fluid (Optiphase Hisafe II LKB). Radioactivity was measured 12 h later in a Packard Tri-Carb 2050 CA counter at 40% efficiency.

Specific binding was defined as the excess over blanks containing 10 μM 5-HT. Protein content was determined by the method of Bradford (1976). Assays were performed in triplicate. Binding affinity (K_d) and receptor number (B_{max}) were determined by linear regression analysis from Scatchard plots. The results were studied statistically by analysis of variance followed by a *t*-test.

Results

[^{3}H]8-OH-DPAT bound in a saturable and reversible manner to cerebral cortex membranes from young and aged rats. Scatchard plots of the data were linear, indicating that [^{3}H]8-OH-DPAT bound in a competitive manner to a single population of sites.

Effect of ageing in control animals. Group means and standard errors for specific [^{3}H]8-OH-DPAT binding parameters are given in Table 1 and Fig. 1 for control cerebral cortex.

Specific [^{3}H]8-OH-DPAT binding was lower in cerebral cortex

Table 1. Affinity of specific [^{3}H]8-OH-DPAT binding (K_d) to cerebral cortex membranes of young and aged rats.

Group	Age (months)	K_d (nmol)
Control	4	2·14±0·24
EGb 761	4	2·18±0·21
Control	24	1·93±0·14
EGb 761	24	2·37±0·21

K_d values were calculated by Scatchard analysis. Each point is the mean of six experiments performed in duplicate or triplicate.

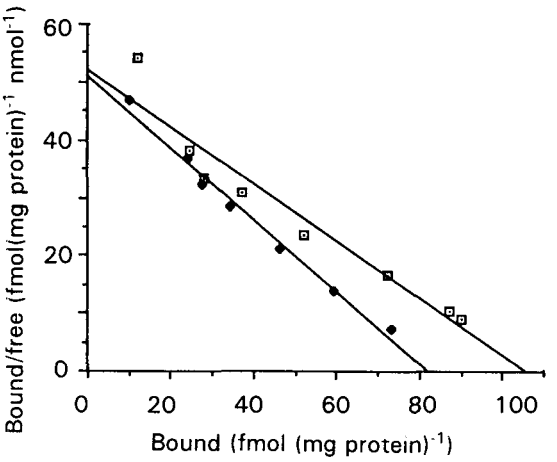

FIG. 1. Scatchard analysis of specific [^{3}H]8-OH-DPAT binding to cerebral cortex membranes of control young and aged rats. Each point is the mean of six experiments performed in duplicate or triplicate. The values of B_{max} were 107·0 ± 5·3 fmol (mg protein)$^{-1}$ for 4-month-old rats (□) and 84·1 ± 5·6 fmol (mg protein)$^{-1}$ for 24-month-old rats (◆) ($P < 0·01$, unpaired t-test).

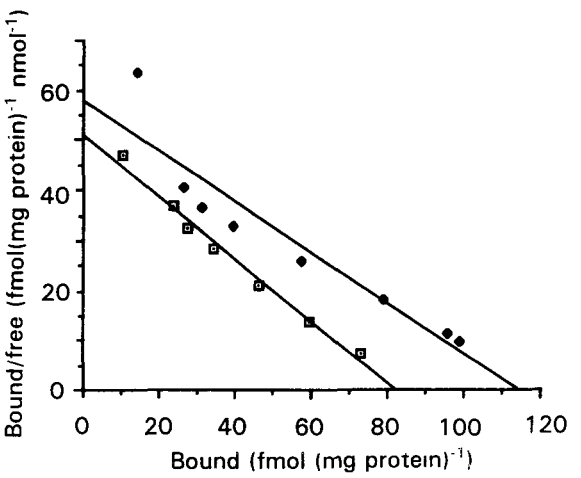

FIG. 3. Scatchard analysis of specific [^{3}H]8-OH-DPAT binding to cerebral cortex membranes of aged rats. Each point is the mean of six experiments performed in duplicate or triplicate. The values of B_{max} were 84·1 ± 5·6 fmol (mg protein)$^{-1}$ for rats treated with vehicle (□) and 112·2 ± 4·2 fmol (mg protein)$^{-1}$ for rats treated with EGb 761 (◆) ($P < 0·005$, unpaired t-test).

membranes obtained from aged rats as compared with young animals. Scatchard analysis showed a significant decrease in the number of [^{3}H]8-OH-DPAT binding sites (B_{max}) in senescent rats. The percentage of difference was −22% ($P < 0·01$). In contrast, binding affinity of [^{3}H]8-OH-DPAT (K_d) did not significantly differ between aged and young rats.

Effect of EGb treatment. Group means and standard errors for the specific [^{3}H]8-OH-DPAT parameters are given in Table 1 and Figs 2 and 3 for placebo and EGb 761 groups.

For the maximum specific [^{3}H]8-OH-DPAT binding (B_{max}), two-way analysis of variance yielded a significant age effect ($F(1,20) = 4·81$, $P < 0·005$), a significant treatment effect ($F(1,20) = 10·52$, $P < 0·005$) and a significant age-treatment interaction ($F(1,20) = 6·41$, $P < 0·02$). This interaction showed that EGb 761 treatment acted differently on the cortical membranes in young and in aged rats. Unpaired Student's t-test revealed no difference in the B_{max} values in the cerebral cortex of young rats between vehicle and the EGb 761 group, but a significant increase (+33%, $P < 0·005$) in aged rats in EGb 761 when compared with vehicle group.

For the affinity of specific [^{3}H]8-OH-DPAT binding (K_d), two-way analysis of variance did not reveal any significant effect and no significant age-treatment interaction. Thus, EGb 761 treatment did not modify binding affinity of [^{3}H]8-OH-DPAT in cerebral membrane of young and senescent rats.

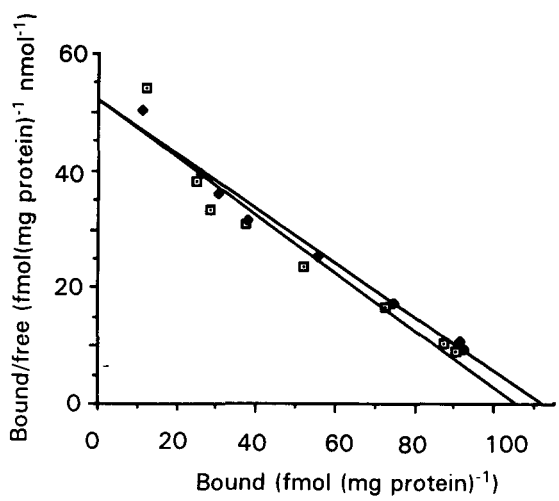

FIG. 2. Scatchard analysis of specific [^{3}H]8-OH-DPAT binding to cerebral cortex membranes of young rats. Each point is the mean of six experiments performed in duplicate or triplicate. The values of B_{max} were 107·0 ± 5·3 fmol (mg protein)$^{-1}$ for rats treated with vehicle (□) and 110·5 ± 4·1 fmol (mg protein)$^{-1}$ for rats treated with EGb 761 (◆).

Discussion

Our experiments showed a decrease in the number of 5-HT_{1A} receptors of the cerebral cortex in senescent rats and a reversal of this change with chronic EGb 761 treatment.

Decreased [^{3}H]8-OH-DPAT binding in aged rats suggests that at least some, if not all, of the age-dependent decrease in 5-HT_1-receptor binding (Petkov et al 1987) is due to changes in the 5-HT_{1A}-receptor subtype. Such an age-related decrease of 5-HT_{1A}-receptor binding density, as measured by autoradiography, was also observed in human cerebral cortex (Dillon et al 1991). These findings indicate that changes in 5-HT_{1A} receptors may reflect changes in the brain that are responsible for the impaired cognition that occurs with ageing. The reduction of the number of 5-HT_{1A} receptors in aged rats is not a compensatory response to 5-HT-ergic activation since both an increase in the 5-hydroxyindoleacetic acid/5-HT ratio (Yeung & Friedman 1991) and a decrease in 5-HT release (Schlicker et al 1989) which indicate a functional reduction of 5-HT-ergic terminals, have been reported in cerebral cortex of senescent rats. In addition, the localization of 5-HT_{1A} receptors on cerebral cortex neurons has been described as postsynaptic (Vergé et al 1986), so that their decrease in senescent rats could not be due to a loss of 5-HT-ergic terminals. The reduction of 5-HT_{1A} receptors and other receptors may result from impaired receptor synthesis (Greenberg 1986) or changes in cerebral neuronal membranes with ageing (Heron et al 1980; Yu et al 1992). In our study, [^{3}H]8-OH-DPAT binding in the cerebral cortex increased after chronic EGb 761 treatment in aged but not young rats. The ability of EGb 761 to enhance 5-HT_{1A}-receptor density may thus be due either to receptor blockade or to induction of indirect receptor hypersensitivity. The first possibility seems unlikely since EGb

761 has only a very low affinity for rat brain 5-HT_{1A}-receptor binding sites (data not shown). However, it has been demonstrated that semi-chronic treatment with EGb 761 increases 5-HT uptake in cerebral cortex synaptosomes (Ramassamy et al 1992), but this effect was obtained at much higher doses than the one used in our study. Taken together, these results suggest that the increase in 5-HT_{1A} receptors after EGb 761 treatment does not result from a modification of synaptic 5-HT-ergic activity since the hypersensitivity of 5-HT_{1A} receptors after EGb 761 treatment was apparent only in the cerebral cortex of aged rats if the density is decreased. Such age-related specificity was recently reported, after the same EGb 761 treatment, on hippocampus α_2-adrenoceptors (Huguet & Tarrade 1992). Similar results were obtained on muscarinic receptors after oral chronic treatment with EGb 761 (Taylor 1986). These findings confirm that EGb 761 does not affect 5-HT-ergic activity specifically and suggest that this extract acts on receptor synthesis or on the membrane of cerebral neurons which are apparently modified with ageing (Heron et al 1980; Greenberg 1986).

EGb 761 has been shown to increase protein synthesis in many cortical regions after unilateral labyrinthectomy in the adult rat (Bustany et al 1992). Thus, in our study EGb 761 treatment might have stimulated the synthesis of synaptosomal plasma membrane protein (e.g. receptors) and might have increased their content which is decreased in old animals (Ragusa et al 1992). Previous data indicate that age-related 5-HT_1-receptor deficit may be associated with age-related changes in neuronal membrane expressed essentially by a decrease in fluidity and an increase in viscosity (Heron et al 1980). It has also been suggested that increased membrane rigidity in aged rats is secondary to peroxidation of membrane fatty acids (Yu et al 1992). In addition, lipid peroxidation reportedly decreases lipid fluidity associated with changes in [^{3}H]5-HT binding (Villacara et al 1989). EGb 761 may block the auto-oxidative membrane process related to lipoperoxide generation and membrane damage (Pincemail & Deby 1986). In fact, flavonoid derivatives are known to exhibit superoxide scavenging activity in-vitro (Robak & Gryglewski 1988), and terpenoid compounds may have antilipoperoxidant activity in-vivo (Pietri et al 1993). These findings suggest that EGb treatment, by inhibiting brain lipid peroxidation, has a restorative effect on age-related decreases in the receptor density of different neurotransmitter systems. Conversely, recent in-vitro studies have demonstrated that EGb 761 protects membrane proteins by preventing the polymerization induced by lipid peroxidation (Dumont et al 1992). The protective effect of EGb 761 on membrane proteins would thus occur subsequently to inhibition of aldehyde production during peroxidative degradation of polyunsaturated fatty acids (Dumont et al 1992). Therefore, this mechanism may also be involved in the restorative effect of EGb on age-related decrease in cerebral receptor density.

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