J. Pharm. Pharmacol. 1992, 44: 24-27
Received March 20, 1991

α_2-Adrenoceptor Changes During Cerebral Ageing. The Effect of *Ginkgo biloba* Extract

FRANÇOIS HUGUET AND THIERRY TARRADE*

*INSERM U. 316, Tours, and Laboratoire de Toxicologie, Faculté de Médecine et de Pharmacie, Poitiers, France, *Institut Henri Beaufour, Paris, France*

Abstract—[^{3}H]Rauwolscine binding to α_2-adrenoceptors in cerebral cortex and hippocampus membranes of young (4 months) and aged (24 months) Wistar rats has been investigated. In aged rats, B_{max} values of [^{3}H]rauwolscine binding were significantly reduced (25–32%) in the cerebral cortex and hippocampus, as compared with the number of α_2-adrenoceptors found in young rats. Chronic treatment with *Ginkgo biloba* extract did not alter [^{3}H]rauwolscine binding in the hippocampus of young rats, but significantly increased (28%) the [^{3}H]rauwolscine binding density in aged rats. These data confirm the previously described age-related noradrenergic alteration and suggest that noradrenergic activity in aged rats is more susceptible to *Ginkgo biloba* extract treatment.

Many functions of the central nervous system change during the ageing process, and several hypotheses have been proposed to explain these age-related disorders. Since neurotransmitter receptors largely govern functional activity of the nervous system, we have studied the influence of age on these receptors, particularly on α-adrenoceptors. In the cerebral cortex, [^{3}H]prazosin binding to α_1-adrenoceptors is identical for young and aged rats (Zhou et al 1984; Carfagna et al 1986; Huguet 1990). However, the recovery of cortical α_1-adrenoceptors is delayed in aged rats after their irreversible inhibition (Zhou et al 1984). In addition, we have shown that cortical α_1-adrenoceptors are upregulated in young, but not in aged rats through a decrease in noradrenergic activity (Huguet 1990). On the other hand, a recent report indicates an age-related increase in presynaptic noradrenergic markers in the rat cerebral cortex (Hedrick & McCracken 1986). Data for cortical α_2-adrenoceptors, localized both pre- and postsynaptically, are inconsistent; results depend on the rat strain and on the ligand used (Kawai et al 1984; Zhou et al 1984; Taylor 1986). Most of those studies were performed with [^{3}H]clonidine, which has also been described as a ligand for imidazoline receptors (Ernsberger et al 1987).

The present study was undertaken to compare α_2-adrenoceptors in the brains of young and aged rats, using the specific ligand [^{3}H]rauwolscine (Diop et al 1983). Moreover, the effect of chronic treatment with *Ginkgo biloba* extract which is widely used in cerebral senescence, on hippocampus α_2-adrenoceptors in young and aged rats was investigated. Several reports describe an effect of *Ginkgo biloba* extract on noradrenergic activity; chronic treatment increases cerebral noradrenaline and decreases β-adrenoceptor number (Brunello et al 1985; Taylor 1986), which may influence ageing.

Materials and Methods

Drugs

The *Ginkgo biloba* extract (GBE 761) was prepared at the Henri Beaufour Institute. It is a well-defined but complex product prepared from green leaves of *Ginkgo biloba*. The leaves are dried and subjected to a 15-step extraction procedure, commencing with an acetone-water mixture under partial vacuum. The final extract is standardized to contain 24% flavonoid glycosides (Ginkgo flavone glycosides) and 6% terpene lactones which are characteristic of *Ginkgo* and have a unique structure (ginkgolides, bilobalide) (Drieu 1986). GBE solution was composed of GBE (50 mg) and mannitol (100 mg) dissolved in 3 mL of distilled water containing 10 mg of disodium phosphate. Vehicle solution was composed of riboflavine (0·1 mg) and mannitol (160 mg) dissolved in 3 mL of distilled water. Solutions were diluted in distilled water.

5-Hydroxytryptamine creatinine sulphate was purchased from Sigma. Guanfacine hydrochloride was donated by Sandoz (Basel, Switzerland). [^{3}H]Rauwolscine (93 Ci mmol^{-1}) was obtained from Amersham (Les Ulis, France).

Animals and treatments

Young (4-month) and aged (24-month) male rats (Wistar, CERJ, Le Genest, France) were housed in groups of 3 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 vehicle were administered i.p. at a dose of 5 mg kg^{-1} once a day for 21 consecutive days in groups of 18 rats.

Membrane preparation

Rats were decapitated 24 h after the last injection of extract or vehicle. Brains were quickly removed and hippocampi were dissected on ice as described previously (Glowinski & Iversen 1966). Tissues were homogenized with Ultra-Turrax (IKA, Labortechnick, T 25) in 10 vol of ice-cold 0·25 M sucrose. The nuclear materials were removed by centrifugation (1000 *g*, 10 min, 4°C) and the supernatants were stored at 4°C. The 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 (pH 7·6) buffer and centrifuged at 50000 *g* for 10 min at 4°C (Beckman J-21C centrifuge). The resultant pellets were

Correspondence: F. Huguet, Laboratoire de Toxicologie, Faculté de Médecine et de Pharmacie, 34 rue du jardin des Plantes, 86034 Poitiers cedex, France.

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 tissue suspension (containing 10 mg wet wt. of original tissue) were incubated with [^{3}H]rauwolscine at concentrations ranging from 0·1 to 15 nM and 5-hydroxytryptamine (5-HT) (1 μM) to inhibit binding to 5-HT-receptors. Incubation was carried out at 25°C in 50 mM Tris-HCl buffer (pH 7·6; final volume 2 mL) for 25 min. The reaction was stopped by a rapid filtration under vacuum through GF/C filters (Whatman). Filters were immediately washed twice with 5 mL of ice-cold buffer and suspended in 7·5 mL of a premixed liquid scintillation fluid (Picofluor 15 Packard). Radioactivity was measured 12 h later in an LKB Wallac 1217 counter at 37% efficiency.

Specific binding was defined as the excess over blanks containing 10 μM guanfacine. The 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 statistically analysed by analysis of variance followed by a *t*-test.

Results

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

Cerebral cortex and hippocampus

Group means and standard errors for the specific [^{3}H]rauwolscine binding parameters are given in Table 1 and Figs 1 and 2 for control (vehicle), cerebral cortex and hippocampus.

For the maximum specific [^{3}H]rauwolscine binding (B_{max}), the 2-way-ANOVA test yielded no age-brain region interaction but a significant brain region effect ($F(1,29)=7{\cdot}81$, $P<0{\cdot}01$) and a significant age effect ($F(1,29)=10{\cdot}69$, $P<0{\cdot}005$). The B_{max} values were slightly greater in hippocampus than in cerebral cortex for both young and aged rats but the differences were not significant. For both cerebral cortex and hippocampus, there was a significant decrease in the number of [^{3}H]rauwolscine binding sites in aged compared with young rats. The percentage of differences were -25 ($P<0{\cdot}01$) and -32 ($P<0{\cdot}02$), respectively.

For the affinity of specific [^{3}H]rauwolscine binding (K_d), the 2-way-ANOVA test yielded no age-brain region interaction but a significant brain region effect ($F(1,29)=51{\cdot}43$, $P<0{\cdot}001$) and a significant age effect ($F(1,29)=7{\cdot}92$, $P<0{\cdot}01$). For both young and aged rats, the binding affinities in cerebral cortex were significantly lower than in hippocampus. There was 63% ($P<0{\cdot}02$) and 65% ($P<0{\cdot}001$) augmentation in young and aged rats, respectively. In cerebral cortex, binding affinity did not differ between aged and young rats. In hippocampus, binding affinity from aged rats was significantly lower than from young rats, with a 48% ($P<0{\cdot}02$) reduction of K_d value.

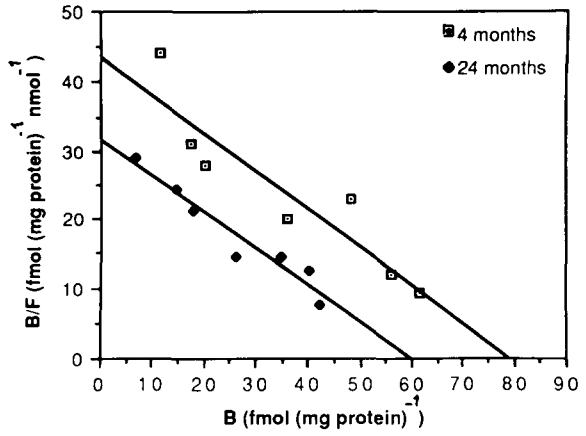

FIG. 1. Scatchard analysis of specific [^{3}H]rauwolscine binding to membranes from cerebral cortex of young and aged rats. Each point is the mean of 5 experiments performed in duplicate or triplicate. The values of B_{max} were $79{\cdot}1 \pm 4{\cdot}4$ fmol (mg protein)$^{-1}$ for 4-month old rats and $59{\cdot}4 \pm 3{\cdot}5$ fmol (mg protein)$^{-1}$ for 24-month old rats and differed significantly ($P<0{\cdot}01$, unpaired *t*-test applied following ANOVA).

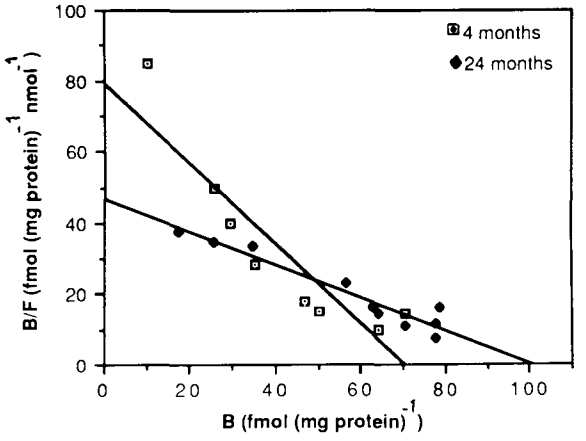

FIG. 2. Scatchard analysis of specific [^{3}H]rauwolscine binding to membranes from hippocampus of young and aged rats. Each point is the mean of 6 experiments performed in duplicate or triplicate. The values of B_{max} were $98{\cdot}4 \pm 9{\cdot}2$ fmol (mg protein)$^{-1}$ for 4-month old rats and $67{\cdot}0 \pm 4{\cdot}5$ fmol (mg protein)$^{-1}$ for 24-month old rats and differed significantly ($P<0{\cdot}02$, unpaired *t*-test applied following ANOVA).

Table 1. Affinity of specific [^{3}H]rauwolscine binding (K_d) to membranes from cerebral cortex and hippocampus of young and aged rats. K_d values were calculated by Scatchard analysis. Each point is the mean of 5 or 6 experiments performed in duplicate or triplicate. [1] $P<0{\cdot}02$, [2] $P<0{\cdot}001$ when compared with the cerebral cortex groups; * $P<0{\cdot}02$ when compared with the 4 months group (unpaired *t*-test applied following analysis of variance).

Brain area	Group	Age (months)	K_d (nmol)	n
Cerebral cortex	Vehicle	4	$2{\cdot}77 \pm 0{\cdot}25$	5
	Vehicle	24	$2{\cdot}53 \pm 0{\cdot}24$	5
Hippocampus	Vehicle	4	$1{\cdot}70 \pm 0{\cdot}25$[1]	6
	GBE	4	$1{\cdot}53 \pm 0{\cdot}16$[2]	6
	Vehicle	24	$0{\cdot}89 \pm 0{\cdot}12$*	5
	GBE	24	$1{\cdot}15 \pm 0{\cdot}22$	6

Effect of GBE treatment

Group means and standard errors for the specific [^{3}H]rauwolscine parameters are given in Table 1 and Figs 3 and 4 for vehicle and GBE groups.

For the maximum specific [^{3}H]rauwolscine binding (B_{max}), the 2-way-ANOVA test yielded no age-treatment interaction but a significant treatment effect ($F(1,29) = 7{\cdot}81$, $P < 0{\cdot}01$). In young rats, there was no difference in the B_{max} values in the hippocampus between vehicle and GBE groups. In aged rats, there was a significant increase (28%, $P < 0{\cdot}05$) in the number of [^{3}H]rauwolscine binding sites in GBE when compared with vehicle groups.

For the affinity of specific [^{3}H]rauwolscine binding (K_d), the 2-way-ANOVA test yielded no age-treatment interaction but a significant treatment effect ($F(1,29) = 4{\cdot}95$, $P < 0{\cdot}05$). K_d values did not significantly differ between GBE and vehicle groups for young and aged rats.

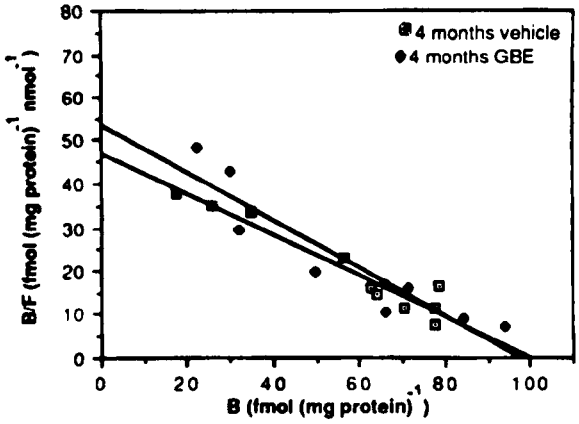

FIG. 3. Scatchard analysis of specific [^{3}H]rauwolscine binding to membranes from hippocampus of young rats. Each point is the mean of 6 experiments performed in duplicate or triplicate. The values of B_{max} were $98{\cdot}4 \pm 9{\cdot}2$ fmol (mg protein)$^{-1}$ for rats treated with vehicle and $92{\cdot}3 \pm 8{\cdot}7$ fmol (mg protein)$^{-1}$ for rats treated with GBE.

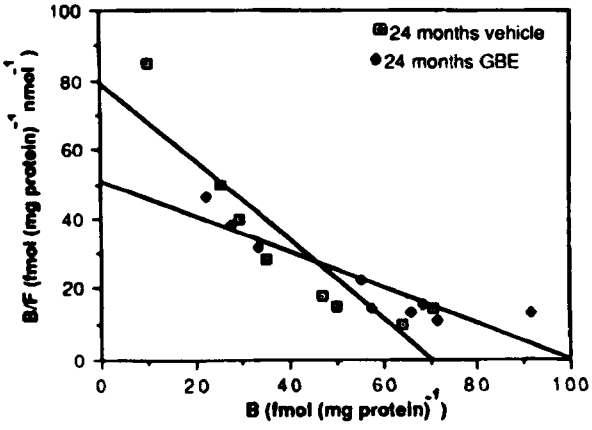

FIG. 4. Scatchard analysis of specific [^{3}H]rauwolscine binding to membranes from hippocampus of aged rats. Each point is the mean of 5 or 6 experiments performed in duplicate or triplicate. The values of B_{max} were $67{\cdot}0 \pm 4{\cdot}5$ fmol (mg protein)$^{-1}$ for rats treated with vehicle and $85{\cdot}5 \pm 6{\cdot}7$ fmol (mg protein)$^{-1}$ for rats treated with GBE and differed significantly ($P < 0{\cdot}05$, unpaired t-test applied following ANOVA).

Discussion

Results of the present experiments show a decrease in number of α_2-adrenoceptors in the cerebral cortex and hippocampus of rat brain during senescence, and up-regulation of α_2-adrenoceptors in the hippocampus of aged rats by chronic GBE treatment.

In the rat brain, [^{3}H]rauwolscine interacts reversibly with stereoselective and high-affinity binding sites, as previously shown (Diop et al 1983). [^{3}H]Rauwolscine identified a large and identical concentration of α_2-adrenoceptors in the cerebral cortex and hippocampus as previously described with [^{3}H]yohimbine, another α_2-adrenoceptor ligand (Bylund 1985). For both ^{3}H-ligands, the binding site density was lower than for [^{3}H]clonidine (Bylund 1985), but [^{3}H]clonidine is also known to bind imidazoline receptors (Ernsberger et al 1987).

Aged animals showed a significant reduction (25–32%) in specific [^{3}H]rauwolscine binding to α_2-adrenoceptors of cerebral cortex and hippocampus membranes. The localization of α_2-adrenoceptors on central neurons of aged rats has been described as being both pre- and postsynaptic (Harik & McCracken 1986; Schlicker et al 1989), however, the present data do not indicate whether the loss of α_2-adrenoceptors observed in aged rats occurred pre- or postsynaptically.

A decrease in α_2-adrenoceptor binding may result from an actual loss or degradation of receptor protein which is not supplied by the synthesis of new receptors for recovery. It is possible that functional inactivation of the receptors may occur due to internalization of the receptor protein (Hollenberg 1985).

[^{3}H]Rauwolscine binding in the hippocampus increased after chronic GBE treatment in aged but not in young rats. The ability of GBE to enhance α_2-adrenoceptor density pre- or postsynaptically may have either been due to a receptor blocking effect or to the induction of indirect receptor hypersensitivity. The finding that chronic yohimbine treatment increases [^{3}H]p-aminoclonidine binding in the cerebral cortex has been shown previously (Swann et al 1981). This seemed unlikely in our study because GBE had only a weak affinity for rat brain α_2-adrenoceptor binding sites (data not shown). On the other hand, it has been shown that chronic GBE treatment elicits a marked increase in normetanephrine in the brain of young rats (Brunello et al 1985). Taken together, these results suggest that the modified α_2-adrenoceptors are presynaptic, thus increasing the negative-feedback inhibition of noradrenaline release and decreasing the excess noradrenaline.

The hypersensitivity of α_2-adrenoceptors after GBE treatment only appeared in the hippocampus of aged rats. A possible explanation for the age-related difference is that aged rats showed both an increase in presynaptic noradrenergic activity and a decrease in α_2-adrenoceptors before GBE treatment as compared with young rats. This suggests that the α_2-adrenoceptors involved in the control of noradrenergic activity are more sensitive to chronic GBE treatment in aged than in young rats. Furthermore, recent studies have demonstrated that chronic GBE treatment induces a decrease in cortical β-adrenoceptors in mature and aged rats (Brunello et al 1985; Taylor 1986). Other experiments have suggested that cortical α_2-adrenoceptors are regulated by β-

adrenoceptors (Maggi et al 1980; Nomura et al 1984). This hypothesis is supported by pharmacological findings that repeated administration of an antidepressant drug, desipramine, causes an increase in α_2-adrenoceptor density and a reduction in β-adrenoceptor density in the rat cerebral cortex (Johnson et al 1980; Asakura et al 1982). Chronic desipramine treatment also has been reported to increase the cerebral noradrenaline level (Bareggi et al 1978). These data suggest that central neurochemical effects obtained after chronic GBE treatment in aged rats are the same as for those obtained after chronic desipramine treatment in mature rats. For both replicate treatments, the consecutive biochemical events were (1) an increase in noradrenaline level, (2) a decrease in β-adrenoceptors, and (3) an increase in α_2-adrenoceptors.

The majority of brain α_2-adrenoceptors do not appear to be located on noradrenergic terminals (Dausse et al 1982). It is conceivable that the α_2-adrenoceptor population which interferes with the β-adrenoceptors is located on the same postsynaptic membrane. β- and α_2-Adrenoceptor interactions were results from direct coupling of β- to α_2-adrenoceptor complexes or indirect involvement of the GTP regulatory protein (Nomura et al 1984).

Published data indicate a significant decrease in brain protein synthesis with age. Hence, the increase of [^{3}H]rauwolscine binding sites observed in aged rats might result from reactivation or reinsertion of the receptors into the membrane. Such phenomena do not seem to involve protein synthesis (Harden 1983).

References

Asakura, M., Tsukamoto, T., Hasegawa, K. (1982) Modulation of rat brain α_2- and β-adrenergic receptor sensitivity following long-term treatment with antidepressant. Brain Res. 235: 192–197

Bareggi, S. R., Markey, K., Genovese, E. (1978) Effects of single and multiple doses of desipramine (DMI) on endogenous levels of 3-methoxy-4-hydroxy-phenylglycol-sulfate (MOPEG-SO4) in rat brain. Eur. J. Pharmacol. 50: 301–306

Bradford, M. N. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254

Brunello, N., Racagni, G., Clostre, F., Drieu, K., Braquet, P. (1985) Effect of an extract of *Ginkgo biloba* on noradrenergic systems of rat cerebral cortex. Pharmacol. Res. Commun. 17: 1063–1072

Bylund, D. B. (1985) Heterogeneity of alpha-2 adrenergic receptors. Pharmacol. Biochem. Behav. 22: 835–843

Carfagna, N., Caccia, C., Sammartini, U., Moretti, A. (1986) Brain aging and turnover of biogenic amines in rats. Gerontology 32 (Suppl. 1): 108

Dausse, J. P., Le Quan-Bui, K. H., Meyer, P. (1982) Alpha1- and alpha2-adrenoceptors in rat cerebral cortex: effect of neonatal treatment with 6-hydroxydopamine. Eur. J. Pharmacol. 78: 15–20

Diop, L., Dausse, J. P., Meyer, P. (1983) Specific binding of ^{3}H-rauwolscine to α_2-adrenoceptors in rat cerebral cortex: comparison between crude and synaptosomal plasma membranes. J. Neurochem. 41: 710–715

Drieu, K. (1986) Préparation et définition de l'extrait de *Ginkgo biloba*. Presse Med. 15: 1455–1457

Ernsberger, P., Meeley, M.P., Mann, J. J., Reiss, D. J. (1987) Clonidine binds to imadazole binding sites as well as alpha$_2$-adrenoceptors in the ventrolateral medulla. Eur. J. Pharmacol. 134: 1–13

Glowinski, J., Iversen, L. L. (1966) Regional studies of catecholamines in the rat brain. J. Neurochem. 13: 655–659

Harden, T. K. (1983) Agonist-induced densensitization of the beta-adrenergic receptor-linked adenylate cyclase. Pharmacol. Rev. 35: 5–32

Harik, S. I., McCracken, K. A. (1986) Age-related increase in presynaptic markers of the rat cerebral cortex. Brain Res. 381: 125–130

Hollenberg, M. D. (1985) Examples of homospecific and heterospecific receptor regulation. Trends Pharmacol. Sci. 6: 242–245

Huguet, F. (1990) Effect of hypertension and age on α_1-adrenoceptors in rat brain: modulation of (^{3}H)prazosin binding by chronic treatment with desipramine and AR-C239. Arch. Int. Pharmacol. Ther. 305: 5–13

Johnson, R. W., Reinstein, T., Spotnitz, S., Wiech, N., Ursillo, R., Yamamura, H. I. (1980) Effects of desipramine and yohimbine on α_2- and β-adrenoceptor sensitivity. Eur. J. Pharmacol. 67: 123–127

Kawai, M., Sakaue, T., Watanabe, C., Nomura, Y., Segawa, T. (1984) Specific binding of [^{3}H]WB 4101, [^{3}H]clonidine and [^{3}H]dihydroalprenolol in cerebral cortical membranes in developing, adult and old rats. Japan J. Pharmacol. 36: 265–267

Maggi, A., U'Prichard, D.C., Enna, S.J. (1980) β-Adrenergic regulation of α_2-adrenergic receptors in the central nervous system. Science 207: 645–647

Nomura, Y., Kawai, M., Segawa, T. (1984) The interaction between β- and α_2-adrenoceptors in cerebral cortical membranes: isoproterenol-induced increase in [^{3}H]clonidine binding in rats. Brain Res. 302: 101–109

Schlicker, E., Betz, R., Göthert, M. (1989) Investigation into the age-dependence of release of serotonin and noradrenaline in the rat brain cortex and of autoreceptor-mediated modulation of release. Neuropharmacology 28: 811–815

Swann, A.C., Grant, S.E., Hattox, S.E. Maas, J.W. (1981) Adrenoceptor regulation in rat brain: chronic effects of α_1- or α_2-receptor blockers. Eur. J. Pharmacol. 73: 301–305

Taylor, J.E. (1986) Liaison des neuromédiateurs à leurs récepteurs dans la cerveau de rats. Effets de l'administration chronique de *Ginkgo biloba*. La Presse Médicale. 15: 1491–1493

Zhou, L.W., Weiss, B., Freilich, J.S., Greenberg, L.H. (1984) Impaired recovery of alpha$_1$- and alpha$_2$-adrenergic receptors in brain tissue of aged rats. J. Gerontol. 39: 538–546