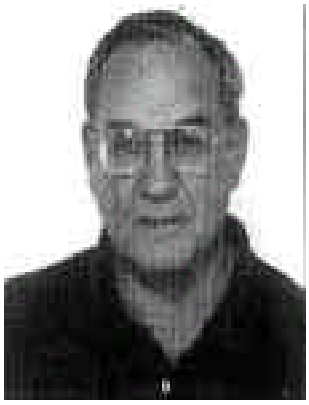
Ginkgo Biloba Extract (EGb 761) and CNS Functions: Basic Studies and Clinical Applications



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Abstract: The effects of EGb 761 on the CNS underlie one of its major therapeutic indications; i.e., individuals suffering from deteriorating cerebral mechanisms related to age-associated impairments of memory, attention and other cognitive functions. EGb 761 is currently used as symptomatic treatment for cerebral insufficiency that occurs during normal ageing or which may be due to degenerative dementia, vascular dementia or mixed forms of both, and for neurosensory disturbances. Depressive symptoms of patients with Alzheimer's disease (AD) and aged non-Alzheimer patients may also respond to treatment with EGb 761 since this extract has an "anti-stress" effect. Basic and clinical studies, conducted both in vitro and in vivo, support these beneficial neuroprotective effects of EGb 761. EGb 761 has several major actions; it enhances cognition, improves blood rheology and tissue metabolism, and opposes the detrimental effects of ischaemia. Several mechanisms of action are useful in explaining how EGb 761 benefits patients with AD and other age-related, neurodegenerative disorders. In animals, EGb 761 possesses antioxidant and free radical-scavenging activities, it reverses age-related losses in brain α_1-adrenergic,5-HT_{1A} and muscarinic receptors, protects against ischaemic neuronal death, preserves the function of the hippocampal mossy fiber system, increases hippocampal high-affinity choline uptake, inhibits the down-regulation of hippocampal glucocorticoid receptors, enhances neuronal plasticity, and counteracts the cognitive deficits that follow stress or traumatic brain injury. Identified chemical constituents of EGb 761 have been associated with certain actions. Both flavonoid and ginkgolide constituents are involved in the free radical-scavenging and antioxidant effects of EGb 761 which decrease tissue levels of reactive oxygen species (ROS) and inhibit membrane lipid peroxidation. Regarding EGb 761-induced regulation of cerebral glucose utilization, bilobalide increases the respiratory control ratio of mitochondria by protecting against uncoupling of oxidative phosphorylation, thereby increasing ATP levels, a result that is supported by the finding that bilobalide increases the expression of the mitochondrial DNA-encoded COX III subunit of cytochrome oxidase. With regard to its "anti-stress" effect, EGb 761 acts via its ginkgolide constituents to decrease the expression of the peripheral benzodiazepine receptor (PBR) of the adrenal cortex.

INTRODUCTION

The Ginkgo biloba tree is described as a "living fossil" since it represents the only surviving species of the order Ginkgoales (class Gymnospermae) that existed when the dinosaurs roamed the earth more than 200 million years ago. The first publication concerning the internal use of the leaves of the Ginkgo tree for medical purposes dates back to 1505 A.D. in a text by Liu Wen-Tai, Ben Cao Pin Hue Jing Yaor. Modern Chinese pharmacopoeias introduced Ginkgo leaves for treating dysfunctions of the heart and lungs [1], and currently extracts of these leaves represent one of the most common phytomedicines in the world. Studies concerning the rigorously standardized extract of Ginkgo biloba leaves, code-named "EGb 761", will be discussed below. This extract contains 24% flavonol glycosides, 6% terpene lactones, and controlled amounts of other substances, including proanthocyanidins and organic acids [2,3].

A polyvalent action of EGb 761 is likely responsible for its efficacy in treating clinical disorders of multifactorial origin. Sub-fractions or various chemical constituents of EGb 761, although active in pharmacological models, do not generally reproduce the actions of the total extract. Thus, additive, antagonistic and synergistic effects of the various active constituents of EGb 761 probably occur with respect to diverse molecular target sites

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in different organs and tissues. Free radical-scavenging/antioxidant activities, membrane receptor interactions and enzyme-inhibition are among the mechanisms that must be considered in efforts to explain the pharmacological and therapeutic actions of EGb 761. Today, some of the mechanisms underlying these actions are being related to certain specific chemical constituents of the extract, but caution must be exercised when such mechanisms are demonstrated in in vitro experiments since the bioavailability of the active substance after oral or parenteral administration must also be established.

The flavonoid constituents of EGb 761 are essentially flavonol-O-glycosides, the glycosidic linkage normally being located in position 3 or 7 of a phenolic aglycon (quercetin, kaempferol or isorhamnetin) and the carbohydrate moiety usually being D-glucose, L-rhamnose or glucorhamnose [3,4]. Such substances act as antioxidants/free radical-scavengers, enzyme-inhibitors and cation-chelators [5]. Certain flavonol glycosides, and/or their metabolites, may play key roles in the therapeutic actions of EGb 761, but their bioavailability after oral administration may well be a limiting factor (see below). The pharmacokinetic properties of flavonoid aglycons are not particularly relevant to EGb 761 since these are present in only trace amounts, if at all, in the extract. However, after oral administration of EGb 761, they could be formed by gastrointestinal microorganisms that metabolize flavonoid glycosides [6].

Flavonoids that traverse the intestinal mucosa of animals are metabolized mainly in cells of the liver after their transport to this organ via the portal vein [7]. The liver possesses the capacity to modify flavonoids and their metabolites and can produce conjugated derivatives [8]. Such conjugated derivatives of flavonoids have been detected in the plasma of rats that were fed rutin [8], which is a constituent of EGb 761. Also, labelled flavonoid metabolites (e.g., 3,5-dihydroxyphenylacetate and 3-hydroxyphenylacetate) have been detected in urine [9], and certain flavonoid metabolites formed during excretion in the bile (via bacterial enzymatic action in the colon) may be reabsorbed and could appear in the blood [10]. Hence, it is expected that certain metabolites of the flavonoid constituents of EGb 761 are absorbed after oral administration of the total extract. A very recent study has shown that only trace amounts of kaempferol (partly in unconjugated form, but mainly as its glucuronide) and a trace amount of free quercetin could be found in urine samples taken from human subjects who had ingested 16 Ginkgo biloba tablets (12.5 mg flavonoids/tablet) over a 48-hour period [11]. Thus, the levels of quercetin and kaempferol found in urine samples were only a very small fraction of the amount ingested.

Pietta *et al.* [12] have recently shown that metabolites of flavonoid glycosides are formed after oral administration of a very high dose of EGb 761 (~ 4 g/kg) to female rats. Flavonoid glycosides, flavonoid aglycons and their metabolites were separated by reversed phase HPLC and identified on the basis of their UV and mass spectra. No glycosides or aglycons were detected in urine, feces or blood, and extensive degradation of flavonoids occurred within 24 hours. Among the seven different phenylalkyl acids that were detected, 3,4-dihydroxyphenylacetic acid (DOPAC; a known dopamine metabolite), benzoylglycine (hippuric acid), 3-hydroxyphenylacetic acid, homovanillic acid (4-hydroxy-3-methoxybenzeneacetic acid) and benzoic acid were directly confirmed by on-line mass spectrometry. Two other substances were identified as 3-(4-hydroxyphenyl)propionic acid and 3-(3-hydroxyphenyl)propionic acid. Absorption was incomplete. Metabolites found in urine represented less than 40% of the flavonoids administered, whereas phenylalkyl acids present in feces were less than 4%. No intact flavonoids were found in blood during 0-5 hours after oral ingestion of the extract, whereas 3,4-dihydroxyphenylacetic acid, homovanillic acid, 3-(4-hydroxyphenyl)propionic acid and 3-(3-hydroxyphenyl)propionic acid (likely to be flavonoid metabolites) could be detected in blood and therefore were bioavailable.

In a further study, in which EGb 761 was given to healthy human volunteers, urine samples contained detectable amounts of substituted benzoic acids, i.e., 4-hydroxybenzoic acid conjugate, 4-hydroxyhippuric acid, 3-methoxy-4-hydroxyhippuric acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, hippuric acid and 3-methoxy-4-hydroxybenzoic acid (vanillic acid) [13]. In contrast to results obtained with rats, no phenylacetic acid or phenylpropionic acid derivatives were found in human urine, indicating that flavonoids are more extensively metabolized in humans. No metabolites were detected in blood samples.

Taken together, these results indicate that the flavonoid constituents of orally administered EGb 761 are generally absorbed in the form of metabolites (phenolic acids) after their transformation by the intestinal flora; see also [14]. A new method based on gas chromatography/negative ion chemical ionization

mass spectrometry of the trimethysilyl derivatives of flavonoids (detection limit ~ 20 pg) has been useful in characterizing the complex mixture of glycosides present in Ginkgo biloba tablets and the trace amounts of flavonoids in urine after ingestion of the extract [11], and could be used for further pharmacokinetic studies; see also [15].

EGb 761 also contains a class of flavonoids termed "proanthocyanidins" or "condensed tannins" (polymers with molecular weights of about 800-6,000) which are potent free radical-scavengers in vitro and which could therefore account for some of the free radical-scavenging activity of the extract, but their bioavailability after oral administration has not yet been examined in detail and may be a limiting factor.

The terpene trilactone constituents of EGb 761 occur exclusively in the Ginkgo tree. Of these, the ginkgolides are diterpenes and bilobalide is a "pentanorditerpene" [16; H. Jaggy, personal communication, 1995]. Ginkgolides A, B, C and J are constituents of EGb 761, and collectively the ginkgolides A, B and C account for about 3.1% of the total extract. Bilobalide accounts for about 2.9% of the extract. A study conducted with healthy human volunteers has shown that the absolute bioavailabilities of ginkgolides A and B are practically complete, whereas that of ginkgolide C is very low when these substances are administered orally as components of the total EGb 761 preparation [17]. The pharmacokinetic properties of bilobalide were difficult to determine in this latter study, due to its instability, but its absolute bioavailability was about 72% after oral administration of EGb 761. A more recent study has shown that ginkgolides A and B and bilobalide are also bioavailable after administration of single oral doses of EGb 761 to rats [18].

Today, EGb 761 is widely employed to treat symptoms associated with "mild-to-moderate" dementia (e.g., disturbances in vigilance, short-term memory loss), impairments of other cognitive functions associated with ageing and senility, related neurosensory problems (e.g., vertigo, tinnitus) and cerebrovascular insufficiency states [19, 20]. Cognitive and neurosensory impairments that may not be associated with dementia also represent clinical targets for EGb 761. The extract opposes abnormal vascular, neurological, rheological, metabolic and immunological functions.

The present article is centered on basic and clinical studies of the effects of EGb 761 on CNS functions. An effort will be made to describe the interactions of certain constituents of EGb 761 with specific molecular targets and to define how these events may contribute to the therapeutic actions of the extract. Particular emphasis will be devoted to the more recent developments. The reader is also referred to several recent reviews on this subject [3, 21-27].

BASIC STUDIES

In Vitro Experiments

Subcellular Particles

High concentrations of EGb 761 inhibit the uptake of radiolabelled norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT) into synaptosome-enriched fractions of rat brain [28]. K_i values (inhibitor concentrations needed to decrease ligand binding by 50%) for these effects of EGb 761 were 0.12 mg/ml for NE in occipital cortex, 0.21 mg/ml for DA in corpus striatum, and 0.44 mg/ml for 5-HT in frontal cortex. In contrast, very low concentrations of EGb 761 (0.1-1 μg/ml) enhanced the in vitro uptake of NE and DA, but not that of 5-HT [29]. As most conventional antidepressants act by inhibiting the presynaptic uptake of biogenic amines, it might be considered that such inhibitory actions of EGb 761 signify antidepressant activity, but the high concentrations of the extract that were required to demonstrate this effect and its lack of specificity imply that this action is of questionable in vivo significance.

Other experiments, conducted with a synaptosomal fraction of mouse cerebral cortex showed that lower concentrations of EGb 761 modified 5-HT uptake in a biphasic fashion; at 4-16 μg EGb 761/ml, uptake was significantly increased, whereas at 32 μg EGb 761/ml and higher, uptake was decreased [30]. This latter effect is probably not specific, since it also occurred with choline and DA uptake, but the increase in 5-HT uptake promoted by the lower concentrations of EGb 761 could be therapeutically relevant since such an effect was also apparent in ex vivo experiments after the animals had been treated orally with EGb 761. In this regard, it seems noteworthy that the atypical antidepressant tianeptine, like EGb 761, increases 5-HT uptake under ex vivo or in vivo conditions [31].

In vitro experiments have also revealed that a mixture of the ginkgolides A, B and C in the ratio of 2:2:1 (0.25 and 10 μg/ml) did not increase synaptosomal 5-HT uptake, but that this uptake was increased by an extract corresponding to EGb

761 but devoid of terpene trilactone constituents (CP 205; 1.25 and 5 μg/ml) [30]. Flavonoid glycosides and/or proanthocyanidins of EGb 761 may have been responsible for this effect. [Note: "CP 205" will be used throughout this article with respect to studies conducted with either "CP 202" or "CP 205" since these two products are identical].

Other experiments have revealed that free radicals generated by ascorbic acid/Fe^{2+}, via peroxidation of membrane lipids, can decrease synaptosomal membrane fluidity and modify the functions of the DA- and 5-HT-transporters, and that EGb 761 can counteract these effects [32,33]. Repeated in vivo treatment of ageing mice with EGb 761 have also been shown to increase brain membrane fluidity [34; see below). Both flavonoid and terpenoid constituents of EGb 761 may be involved in such actions; i.e., flavonoids via scavenging free radicals and other ROS, and possibly by acting as Fe^{2+}-chelators [32,33], and terpenoids via decreasing the generation of free radicals [35].

Apoptosis in Cell Cultures

Flow cytometric and DNA fragmentation analyses of primary cultures of cerebellar granule cells prepared from 7-day-old rats have been used to examine the effect EGb 761 on neuronal damage induced by oxidative stress [exposure of the cells to hydrogen peroxide (H_2O_2) + ferrous sulfate, which leads to the formation of hydroxyl radicals ($OH^{\bullet}$)] [36]. Pretreatment of the cells with EGb 761 (100 μg/ml) decreased $OH^{\bullet}$-induced apoptosis ("programmed cell death"), an effect that likely involved the free radical-scavenging and anti-lipoperoxidative actions of the extract. More recent experiments have shown that the total EGb 761 preparation, one of its flavonoid constituents (rutin), as well as a mixture of flavonoids and terpenoids, protected cultured cerebellar granule cells against oxidative damage and apoptosis induced by $OH^{\bullet}$, whereas the total terpene fraction of EGb 761 did not protect against apoptosis [37].

With further regard to apoptosis, the effects of EGb 761, ginkgolide B and bilobalide have been assessed on cell viability, the incidence of spontaneous apoptosis, and the incidence of apoptosis induced by a peroxyl radical-generator (2,2'-azobis 2 amidinopropane; AAPH) using primary cultures of hippocampal nerve cells [38]. Addition of AAPH (20 or 50 mM) to the medium decreased cell viability and increased the number of apoptotic cells. Addition of EGb 761 (5-20 μg/ml) or ginkgolide B (0.2 or 0.4 μg/ml) to the culture medium increased cell viability and decreased the number of hippocampal cells undergoing AAPH-induced apoptosis, whereas addition of bilobalide (0.1-1.0 μg/ml) was ineffective. Repeated (8-day) oral administration of EGb 761 and ginkgolide B (but not bilobalide) to rats in vivo significantly increased cell viability and reduced both spontaneously-occurring and AAPH-induced apoptoses, indicating that the in vivo effects were more potent than those observed in vitro. As AAPH enhances the production of peroxyl radicals, these protective actions of in vivo treatments with EGb 761 and ginkgolide B appear to be associated with anti-lipoperoxidative activity.

It has also been recently reported that EGb 761 and some of its constituents can inhibit the neuronal apoptosis that is induced in cultured chick embryonic neurones and in mixed cultures of neurones and astrocytes from neonatal rat hippocampus either by serum deprivation or by staurosporine, both of which lead to increased generation of ROS [39]. In this study, the increase in the percentage of apoptotic chick neurones that followed 24 hours of serum deprivation was reduced to control level by EGb 761 (10 mg/l), ginkgolide B (10 μM), ginkgolide J (100 μM) and bilobalide (1 μM). Apoptosis of chick neurones induced by a 24-hour exposure to staurosporine (200 nM) was also found to be significantly reduced in the presence of EGb 761 (100 mg/l), ginkgolide J (100 μM) and ginkgolide B (10 μM), and bilobalide (10 μM) decreased apoptotic damage induced by a 12-hour staurosporine treatment to nearly the control level. In mixed neuronal/glial cultures, EGb 761 (100 mg/l) and bilobalide (100 μM) protected rat neurones against apoptosis caused by serum deprivation, and bilobalide (100 μM) and ginkgolide B (100 μM) reduced staurosporine-induced apoptotic damage, whereas ginkgolide A had no anti-apoptotic effect in either serum-deprived or staurosporine-treated neurones. Taken together, these results confirmed and extended an earlier study [40] which showed that sectioning of the olfactory nerve induced apoptosis in adult rats and that the rate of this apoptosis was reduced by pretreatment of the animals with EGb 761. These workers [39] believe that EGb 761 and some of its constituents possess anti-apoptotic activity and that bilobalide is the most potent constituent.

Certain discrepancies are obvious upon comparing the results of the above-mentioned studies. Two of the studies have led to the conclusion that terpenoid constituents of EGb 761 can counteract (prevent) apoptosis [38,39],

whereas another study indicates that the terpenoid constituents of EGb 761 do not influence apoptosis [37]. Also, upon comparing the studies in which terpenoid constituents were active, one study revealed that ginkgolide B, but not bilobalide, was effective in opposing apoptosis [38], whereas another study indicated that bilobalide was the most potent anti-apoptotic EGb 761 constituent tested [40]. These discrepancies could be related to the use of different types of cultured cells and/or to other differences in the methodologies employed by these three research groups. In this regard, the in vitro experiments of Rapin *et al.* [38] were conducted with lyophilized soluble formulations of ginkgolide B and bilobalide prepared by a procedure that involves partial opening and salinization of their lactone rings (a type of "salting-in" procedure), whereas ginkgolides or bilobalide that had been dissolved in dimethyl sulfoxide (DMSO; which possesses various pharmacological activities that confound interpretation of experimental results) were generally used in other studies [37,39]. These preparations of ginkgolides would be expected to have different activities. Also, a relative instability of bilobalide under in vitro conditions (half-life ~3.5 hr at pH ~7.0) could contribute to the different results obtained with this terpene trilactone.

Regardless of the explanation, it is evident that results concerning the possible anti-apoptotic roles of the active constituents of EGb 761 require confirmation, especially since an anti-apoptotic effect of EGb 761 would likely represent an effect of opposing oxidative damage. Since apoptosis is intimately linked to oxidative stress and since oxidative stress appears to be a major process involved in AD pathology, further clarification of the EGb 761 constituents that have anti-apoptotic effects could be relevant to explaining the beneficial effects of the extract in treating AD, as well as its possible benefits for treating other neurodegenerative and ischaemic disorders of the CNS whose pathogenesis may involve oxidative stress and apoptosis [41,42].

Cell Cultures and Nitric Oxide

Other recent studies, performed with cell cultures, have focussed on cell damage that can be induced by nitric oxide (NO). In addition to the beneficial actions of NO on the cardiovascular system, it is well known that excessive NO production can lead to deleterious effects [43,44]. The mechanism of such destructive effects of NO probably involves its reaction with $O_2^{-\bullet}$ which leads to the formation of peroxynitrite, and then to enhanced generation of highly detrimental $OH^{\bullet}$ (see also [45]).

As EGb 761 is a free radical-scavenger, Goureau and Courtois [46] have tested its ability to inhibit the deleterious effects of excessive NO production in bovine retinal pigmented epithelial (RPE) cells. Incubation of RPE cell cultures with the EGb 761, together with lipopolysaccharide and interferon-γ (the latter two substances being used to increase the expression of inducible NO-synthase), led to a decrease in nitrite release while not modifying citrulline synthesis. It was proposed that EGb 761 acted by scavenging the free radical form of NO ($^{\bullet}N=O$) in producing this beneficial effect, and that this action of EGb 761 could protect the retina from the endotoxin- and cytokine-mediated damage that occurs during ocular pathology and ageing. Other experiments, conducted with a hippocampal cell-culture model (derived from fetal rats) have shown that EGb 761 prevents cell death induced by pro-oxidants such as H_2O_2 and sodium nitroprusside/NO [47]. Such protection probably involves $OH^{\bullet}$- and NO-scavenging activities of EGb 761 which are mediated by its flavonoid constituents [see 21].

Oxidative Phosphorylation and Mitochondrial Respiration

Experiments performed with cell cultures have also revealed that bilobalide can regulate oxidative phosphorylation. Human mitochondrial DNA (mtDNA) encodes 13 polypeptides, among which are the COX I, COX II, COX III subunits of cytochrome c oxidase (COX). The activity of COX and the levels of COX subunit mRNAs can be considered to be markers of oxidative metabolism and neuronal activity, and therefore the molecular mechanism by which EGb 761 protects neurones from ischaemia could be associated with stimulation of mitochondrial gene expression. To test this hypothesis, Chandrasekaran *et al.* [48] used a variant of rat pheochromocytoma PC12 cells termed "PC12S", which differentiate in the presence of nerve growth factor (NGF) and whose morphology resembles that of sympathetic neurones. [The expression of mtDNA-encoded genes can be regulated by changes in intracellular Na^+, and addition of ouabain (an inhibitor of Na^+/K^+-ATPase) causes a significant decrease in the level of the mtDNA-encoded COX III gene.] This system was used to determine the possible effects of ginkgolide B and bilobalide on the level of mtDNA-encoded COX III mRNA, and their possible protective effect against ouabain-induced decreases in mitochondrial gene expression. No significant

change in the levels of COX III mRNA occurred when PC12S cells were differentiated with NGF for 10 days and then treated with vehicle (controls) or exposed to ginkgolide B (0.2-10 μg/ml) for 6 hours (Northern blot analyses of total RNA). However, bilobalide (0.2-10 μg/ml) caused a significant increase in the ratio of COX III mRNA to β-actin mRNA (a nuclear DNA-encoded control substance), the increase being two-fold at the highest concentrations tested (5 and 10 μg/ml). Other experiments showed that 15-minute or 24-hour pretreatment of differentiated cells with bilobalide (10 μg/ml) significantly protected against ouabain-induced decreases in COX III mRNA. Although these results are limited in that they were obtained with a single cell culture system, they do show clearly that bilobalide influences COX III gene expression. An element of specificity is evident since ginkgolide B did not influence COX III gene expression or the action of ouabain.

The finding that bilobalide stimulates mtDNA-encoded COX III mRNA is consistent with results obtained with other models. In vivo administration of bilobalide increased "state 3" mitochondrial respiration (maximal rate of respiration achieved in the presence of substrate, ADP and P_i) following cerebral ischaemia in gerbils [49]. Bilobalide inhibited hypoxia-induced decreases in ATP content in vascular endothelial cells in vitro, and in an ex vivo model the respiratory control ratio (RCR; the ratio between state 3 which is the rate of phosphorylating respiration in the presence of exogenous ADP, and state 4 which is the rate of respiration when all ADP has been consumed) of liver mitochondria was increased in rats that had been repeatedly treated with bilobalide [50]. More recent results, obtained both in vivo (ex vivo) and in vitro, have revealed that bilobalide protects against ischaemia-induced decreases in state 3 liver mitochondrial respiration by preserving the function of Complexes I and III, thereby permitting the mitochondria to maintain their respiratory activity under ischaemic conditions as long as some oxygen is present [51]. Since ischaemia leads to a loss in the capacity of ATP and ADP concentrations to control the rate of mitochondrial electron transport; i.e., uncouples ATP synthesis from electron transport (uncouples oxidative phosphorylation), this preservation of ATP content by bilobalide is best explained by its protection of oxidative phosphorylation.

These results are also in accord with those obtained in studies of triethyltin- (TET-) and bromethalin-induced cerebral edema (see below). Intoxication with both of these agents uncouples oxidative phosphorylation, inhibits mitochondrial ATPase activity, and leads ultimately to cytotoxic brain edema. The mechanism underlying the beneficial effect of bilobalide in these models of edema is probably associated with its prevention of the uncoupling of oxidative phosphorylation and preservation of ATP levels.

The findings of Chandrasekaran *et al.* [48] support the contention that EGb 761 and its constituent, bilobalide, can be used as therapy for AD and other neurodegenerative diseases. In this regard, Chandrasekaran *et al.* [52,53] have measured the mRNA levels of mtDNA-encoded COX I and III, as well as mtDNA-encoded 12S rRNA in postmortem brains of AD patients and age-matched healthy controls. Brains from AD patients showed a 50-65% decrease in mRNA levels of COX I and III in the middle temporal association neocortex (which contained more neuritic plaques and neurofibrillary tangles than primary motor cortex), but not in the primary motor cortex, as compared to control brains. The amount of mitochondrial-encoded 12S rRNA was not altered, nor was the amount of nuclear-encoded lactate dehydrogenase B mRNA (a marker of glycolytic metabolism). These data, which demonstrated a specific decrease in mitochondrial markers of oxidative metabolism (COX I and COX III mRNAs) in the association but not in the primary neocortical region in AD brains, indicated that the decrease in COX I and III subunit mRNAs in the affected brain regions may contribute to reduced brain oxidative metabolism in AD. Hence, EGb 761 or bilobalide treatment, via a gene-regulatory action on COX subunits, could reverse or prevent the impaired mitochondrial regulation that occurs in AD brain, and which involves excessive generation of ROS and the initiation of apoptosis.

Brain Samples from Alzheimer's Patients and Apolipoprotein E

Inheritance of the ε4 allele of apolipoprotein E (ApoE; a glycoprotein involved in cholesterol homeostasis and lipid turnover) is considered to be a major risk factor for late-onset familial and sporadic forms of AD [54,55]. In an effort to define the possible relationship between ApoE genotype and oxidative stress in AD, Ramassamy *et al.* [56] have examined the effect of EGb 761 on stimulated oxidation of phospholipids in hippocampal and frontal cortical samples from AD patients with different ApoE genotypes. Under in vitro conditions in which oxidation was induced by high concentrations of H_2O_2 and $FeSO_4$, EGb 761

(25-50 μg/ml) completely prevented the induction of (phospho)lipid oxidation in control tissues and in tissues of AD patients with the ε3/ε3 or ε3/ε4 genotype, whereas this protective effect was less potent in tissues of patients with the ε4/ε4 genotype. These and other results, indicated that the brain samples of AD patients possessing the ε4 allele show more aberrant lipid homeostasis and that treatment with EGb 761 can reverse these disturbances.

Comment

Collectively, the findings discussed in this section support continued clinical testing of EGb 761 as therapy for AD, and indicate further that novel therapy for AD, as well as for other neurodegenerative diseases (e.g., Parkinson's disease, amyotrophic lateral sclerosis, Huntington's chorea) and CNS ischaemic disorders, might be developed using bilobalide. EGb 761 may act mainly via its bilobalide constituent to restore or counteract the energy failure of brain neurones that characterizes these disorders.

In Vivo and Ex Vivo Studies

Cerebral Blood Flow and Glucose Utilization

Early studies revealed that treatment with EGb 761 can significantly increase cerebral blood flow (CBF) and cerebral 2-deoxy-D-glucose uptake in experimental animals [see 3]. However, it should be noted that in some cases such increases in CBF were obtained by injecting very high doses of EGb 761 [57]. Other studies conducted with lower doses of EGb 761 have led to the conclusion that treatment with this extract restores (increases) cerebral glucose utilization in animals whose glucose consumption is decreased by ischaemia or normobaric hypoxia, and that it acts oppositely in normal animals, in accord with the brain's metabolic demand [58-60]. Several mechanisms could be associated with these effects of EGb 761; e.g., rheological effects involving platelets and erythrocytes, antioxidant effects [see 3,21], increased synthesis of "endothelium-derived relaxing factor" (EDRF; now generally considered to be NO) [61]), and certain peripheral anti-vasoconstrictor effects [62]. This remains as an area open to further investigation.

These effects of EGb 761 could be considered to counteract the decline of energy availability to neurones which leads to their degeneration in AD and other neurodegenerative diseases. In this context, it is known that brain glucose metabolism is impaired in AD patients and in subjects who are genetically at high risk for AD (ApoE4 homozygous) [63]. PET analyses have shown that late middle-aged human subjects who have normal cognition and who are homozygous for the ε4 allele of ApoE have reduced rates of glucose metabolism specifically in the posterior cingulate, parietal, temporal and prefrontal regions of the brain, i.e., the same brain regions as those affected in patients with probable AD. The studies mentioned above [e.g., 59,60] indicate that EGb 761 treatment could counteract these changes since the brain regions in which glucose utilization is reduced in patients with probable AD and in those who are genetically at high risk for AD (ApoE4 homozygous) are among those influenced by EGb 761 treatment in rats.

Collectively, these findings support the contention that EGb 761 might provide effective treatment for AD patients, one of its major targets being the energy failure of brain neurones. As increases in circulating levels of glucocorticoids, via their effects of reducing glucose transport and utilization, may contribute to reduced energy availability and neurodegeneration in the AD brain [see 64], the recent results of Amri *et al.* [65] which showed that repeated EGb 761 treatment can decrease glucocorticoid synthesis are of special interest (see below).

Cerebral ischaemia, Hypoxia and Edema

Numerous studies have shown that treatment of rats with EGb 761 can prevent the neurological deficits and death that may follow cerebral ischaemia [see 3,21]. Beck *et al.* [66] have suggested that these beneficial effects of EGb 761 are associated with its non-flavone (i.e., terpenoid) fraction. Other experiments, performed with gerbil models of cerebral ischaemia, have shown that EGb 761 treatment partially opposed the increases that occur in the water and Na^+ contents and the decrease in K^+ content of the brain [67], and that repeated oral administration of EGb 761 before inducing ischaemia can prevent the "delayed" type of hippocampal neurodegeneration [68]. Such protective effects of EGb 761 may be mediated by its free radical-scavenging/antioxidant and anti-lipoperoxidative activities which likely involve its flavonoid constituents, by the anti-ischaemic activity of ginkgolides [35,69], and by an action of bilobalide of preserving mitochondrial respiratory function [49-51; see above].

The anti-ischaemic effect of EGb 761 is further supported by the finding that oral treatment of

gerbils with ginkgolide B (10 mg/kg/day) for three days significantly protected against the inhibition of hippocampal Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activity that occurred after inducing cerebral ischaemia [70]. These findings are of interest, since the molecular target of ginkgolide B, CaMKII in this case, is associated with brain repair, long-term potentiation in the hippocampus, and mechanisms of learning and memory.

Preventive and/or curative effects of EGb 761 have been observed on cytotoxic brain edema induced by TET, hexachlorophene or bromethalin in rats [71-73] and on trauma-induced vasogenic brain edema in rabbits [74]. In these models, the mechanism underlying the beneficial effect of bilobalide is presumed to involve a preservation of ATP, prevention of uncoupling of oxidative phosphorylation [50,51], and stabilization of membranes against hypoxia-mediated degradation [75]. Such an action of bilobalide could involve, as its molecular target, its up-regulation of the expression of the COX III subunit of cytochrome c oxidase, an oxidative phosphorylation enzyme of the mitochondria [48; see above]. This viewpoint implies that EGb 761 treatment may be useful in treating CNS problems characterized by edema and/or inflammation (e.g., traumatic brain injury; perhaps AD).

Neurotransmitter Systems and Ageing

Repeated oral administration of EGb 761 (100 mg/kg/day) for 28 days increased the binding of [^{3}H]quinuclidinyl benzilate to hippocampal muscarinic cholinergic receptors of aged Fischer-344 rats to the level found in young rats without influencing this binding in young rats [76]. With further regard to central cholinergic systems, administration of EGb 761 (50 mg/kg/day, p.o.) for 30 days significantly increased ex vivo Na^+-dependent high-affinity [^{3}H]choline uptake (HACU) into hippocampal synaptosomes of old (24-month-old) rats [77]. Direct in vitro addition of EGb 761 (15-30 μg/ml) also caused significant increases in HACU. These findings imply that EGb 761 treatment produces a functional activation of cholinergic nerve terminals in the hippocampus in old rats.

The age-related decrease in the density of hippocampal α_2-adrenoceptors of rats, determined by [^{3}H]rauwolscine binding, was significantly reversed by subchronic (21-day) treatment with EGb 761 (5 mg/kg/day, i.p) [78]. The B_{max} value (maximal binding capacity) of [^{3}H]rauwolscine in hippocampal membrane preparations was significantly decreased in 24-month-old rats (as compared to 4-month-old rats), and EGb 761 treatment significantly increased B_{max} in older rats, but did not alter this parameter in young rats. The binding of [^{3}H]8-hydroxy-2(di-n-propylamino)tetralin ([^{3}H]8-OH-DPAT; 0.8-10 nM) to 5-HT_{1A} receptors in cerebral cortical membranes of rats occurred with a B_{max} that was significantly lower in ageing (24-month-old) than in young (4-month-old) animals, whereas binding affinity (reciprocal of K_D) did not differ between the two groups [79]. In this latter study, repeated treatment with EGb 761 (5 mg/kg/day, i.p.) for 21 days did not alter this binding in young or old rats, but significantly

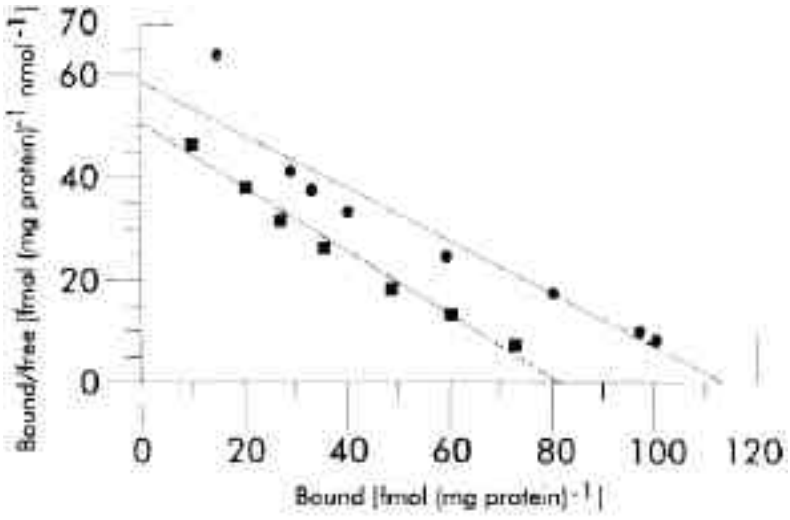


Fig. 1. Scatchard analysis of specific [^{3}H]8-OH-DPAT binding to cerebral cortex membranes of aged rats. Each point is the mean of 6 experiments performed in duplicate or triplicate. Values for B_{max} were 84.1 ± 5.6 fmol/mg protein for rats treated with vehicle (■) and 112.2 ± 4.2 fmol/mg protein for rats treated with EGb 761 (• ; 5 mg/kg/day) for 21 consecutive days ($p < 0.005$, unpaired t-test). (Reproduced with permission from Huguet *et al.* [79]).

increased B_{max} in old rats, as compared to vehicle-treated controls; see Fig. (**1**).

Taylor [76] found that although the B_{max} for β-adrenergic receptor binding to cerebral cortical membranes was lower in old rats, treatment of either young (3-month-old) or old (24-month-old) rats with EGb 761 (100 mg/kg/day, p.o.) for 28 days did not influence β-adrenoceptor binding.

Collectively, these results indicate that EGb 761 has a "restorative effect" on age-related decreases in neurotransmitter receptor density. This effect of EGb 761 may be mediated by its inhibition of lipid peroxidation-induced membrane damage via its free radical-scavenging/antioxidant actions. An effect of restoring the age-related decrease in $5\text{-}HT_{1A}$ receptors is particularly interesting since these receptors exist mainly in brain regions, such as cerebral cortex (neocortex) and hippocampus, that are involved in regulating cognitive functions [80]. Thus, support is gained for using EGb 761 to decelerate brain neurodegeneration and to treat AD and other cognitive disorders of the elderly.

Age-associated Impairment of Rat Brain Mitochondria

Free radical-induced damage impairs mitochondrial function and can cause mutations in mitochondrial DNA that may contribute to age-related neurodegenerative diseases, and therefore antioxidant treatment may oppose such age-related disorders [81].

In line with this hypothesis, an ex vivo study conducted with young (4-month-old) and old (24-month-old) rats has revealed that mitochondrial function under "state 4" conditions (i.e., basal respiratory rate) undergoes an age-related decrease which can be prevented by treatment with EGb 761 (100 mg/kg/day, p.o.) for three months [82]. EGb 761 treatment also partially prevented other age-related indices of oxidative damage, such as the change in the ratio of small to large brain mitochondria, the oxidation of mitochondrial glutathione, mitochondrial peroxide generation, and peroxide release into mitochondria. In a related study [49], repeated administration of EGb 761 (50 mg/kg/day, p.o.) to ageing (18-month-old) Fischer-344 rats did not affect basal respiratory rate (state 4) in mitochondria, but significantly increased mitochondrial "state 3" oxygen uptake after 14 or 21 days. This effect of EGb 761 treatment, which preserved oxidative phosphorylation and ATP synthesis, may have been due to its content of bilobalide [48,50,51; see above] and is useful in explaining the beneficial effects of the extract on cerebral ischaemia, neurodegenerative disorders and age-related impairment of cognitive functions.

Learning, Memory and Synaptic Plasticity

Winter [83] tested the effects of EGb 761 on acquisition, performance and retention in mice using an appetitive operant conditioning paradigm. Administration of EGb 761 (100 mg/kg/day, p.o.) was begun 4 or 8 weeks before training the animals for 30 consecutive days to acquire a two-lever response sequence followed by food reward and was continued until performance of a retention test 10 weeks later. EGb 761 facilitated memory processes, as evidenced by an increased number of "correct responses", more frequent performance of correct responses in the most effective manner, and more rapid reduction of the number of "incorrect responses"; see Fig. (**2**) EGb 761 treatment also improved retrieval of the learned response. The molecular targets underlying these effects of EGb 761 remain unknown, but could be related to effects the extract on central neurotransmitter systems (e.g., muscarinic cholinergic, serotoninergic or noradrenergic receptors or uptake processes) and/or to modulation of cerebral metabolism and blood flow.

Blavet [84] used an eight-arm radial maze to compare the effects of EGb 761 on the performance of 8-, 12- and 18-month-old male Fischer-344 rats. This rat strain ages more rapidly than other strains, a phenomenon that is reflected as deficits in cerebral cortical choline acetyltransferase activity and muscarinic receptor density [85]. Treatment with EGb 761 for three weeks before testing and throughout the testing period enhanced learning parameters in the 12-month-old rats and improved learning in a dose-related manner in 18-month-old rats. Such effects of EGb 761 could be related to its action of increasing muscarinic receptor density in the brain (see above).

Another recent study has shown that repeated treatment of mice with EGb 761 (100 mg/kg/day, p.o.) for three weeks significantly improved short-term memory in a passive avoidance paradigm and increased brain neuronal membrane fluidity (measured as membrane anisotropy) [34]. The effect of EGb 761 on membrane fluidity, which occurred to a significantly greater extent in aged EGb 761-treated animals than in aged controls, might involve its antioxidant and anti-lipoperoxidative actions which could modify the functions of membrane DA- and 5-HT-transporters [32,33; see above]. Other experiments, conducted

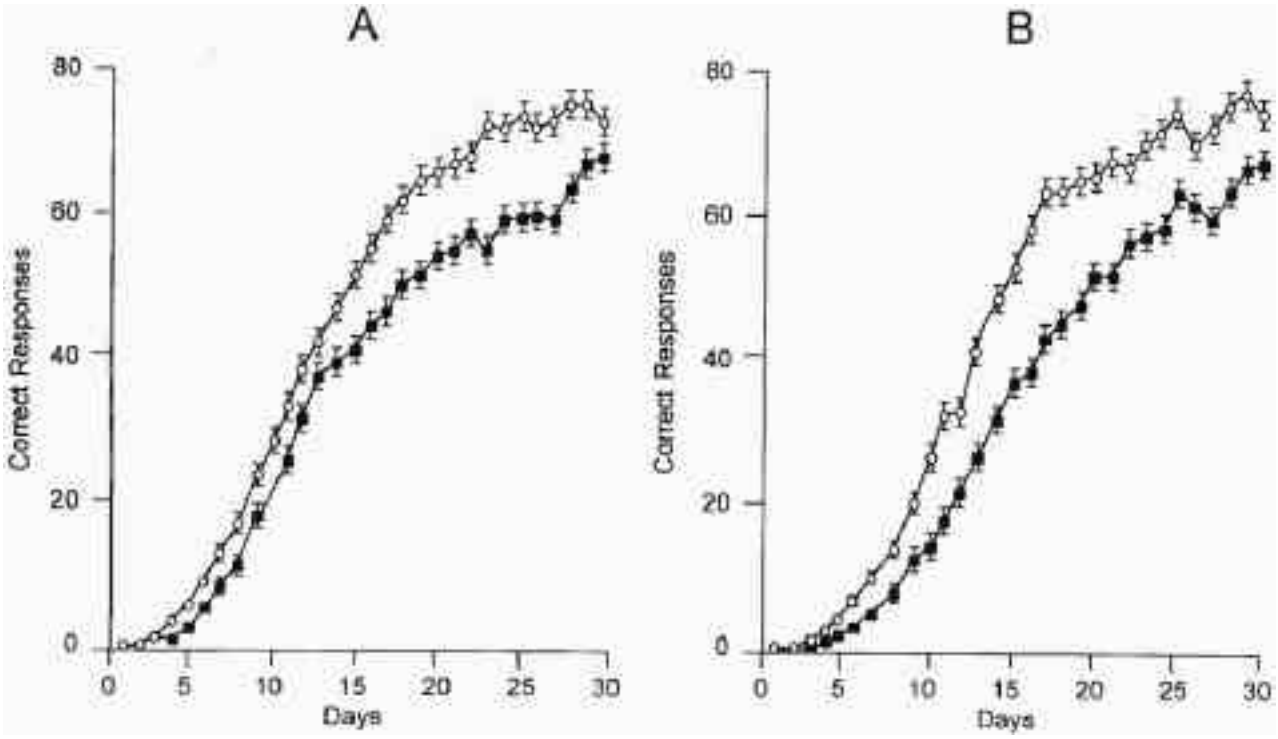


Fig. 2. Effect of EGb 761 on the number of correct responses during 30 consecutive days of lever-press conditioning. Results are expressed as means ± S.E.M. per day.

A: Daily administration of EGb 761 (100 mg/kg) was initiated 4 weeks before the conditioning period. The level of correct responses is significantly increased by EGb 761 ($p < 0.001$). (U) EGb 761 (n = 27); (■) Control (n = 25). B: Daily administration of EGb 761 (100 mg/kg) was initiated 8 weeks before the conditioning period. The level of correct responses is significantly increased by EGb 761 ($p < 0.001$). (U) EGb 761 (n = 18); (■) Control (n = 17). (Reproduced with permission from Winter [83]).

with a rat model of radiation-induced encephalopathy and two negative reinforcement tests given sequentially before and/or after brain irradiation, have shown that treatment of the animals with EGb 761 (50 or 100 mg/kg, p.o.) for 4 consecutive weeks may reduce irradiation-induced behavioral deficits [86]. In these experiments, EGb 761 treatment not only protected against the loss in capacity for retention in these tests, but also enhanced the ability of the animals to learn a new task, this latter effect being evident even three weeks after cessation of treatment. Such results provide a basis for testing the possible therapeutic action of EGb 761 on irradiation-induced cognitive dysfunction.

Most recently, Winter [87] has examined the effects of orally self-administered EGb 761 on "continuous learning" and "delayed non-matching to position" in male Fischer-344 rats using an eight-arm radial maze. Chronic post-session administration of EGb 761 (50 mg/kg) did not affect continuous learning but pre-session administration of this same dose resulted in a propensity toward fewer sessions to reach criterion performance as well as fewer errors. In the "delayed non-matching to position" task, pre-session administration of EGb 761 caused dose-related reductions in total, retroactive, and proactive errors. Pre-session administration of EGb 761 (200 mg/kg) to the rats (at 26 months of age) led to a statistically significant positive treatment effect, as compared to vehicle-treated controls. Interestingly, rats that were chronically treated with EGb 761 lived significantly longer than vehicle-treated subjects. These results are consistent with the beneficial effects of EGb 761 on cognitive performance which have been determined in human subjects.

In a further study, Winter and Timineri [88] established "stimulus control" in a group of nine rats using EGb 761 (10 mg/kg, i.p., 15 min before training). A two-lever operant task with 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 involved the appropriate lever, a mean of 24 sessions was required to reach criterion

performance. Such EGb 761-induced stimulus control was significantly antagonized by the selective 5-HT_{1A} antagonist WAY-100635, but unaffected by the 5-HT_2 antagonist pirenperone. Also, EGb 761 generalized to the selective 5-HT_{1A} agonist, 8-OH-DPAT, an effect that was inhibited by WAY-100635. Taken together, these results indicated that intraperitoneally administered EGb 761 can induce stimulus control by an effect that is mediated in part by an action at 5-HT_{1A} receptors.

With regard to recovery of behavioral function after traumatic brain injury, Attella *et al.* [89] have examined the effects of EGb 761 on behavior before and after bilateral aspiration of the medial frontal cortex or sham operations in rats. Rats bearing lesions that had been treated with EGb 761 (100 mg/kg/day, i.p.) for 30 days were less impaired than saline-treated controls in retention of a delayed spatial alternation task, a finding that correlated with decreased brain edema [see also 90,91]. Although such effects of EGb 761 were modest, they did show that EGb 761 treatment can facilitate recovery from certain cognitive deficits that follow traumatic brain injury.

With further regard to recovery of function, Brailowsky *et al.* [91,92] examined the effects of subacute (7-day) and subchronic (30-day) EGb 761 treatments using two rat models of cortical hemiplegia. The elevated beam test was used to evaluate coordinated walking in water-deprived animals that had been trained to drink saccharin-sweetened solutions (with or without EGb 761) and to perform to criteria before surgical intervention. Rats that received the extract showed a faster and more complete recovery from motor deficits than those which received only saccharine solutions, whereas no differences were detected for sensory deficits. A comparison of the effects of authentic EGb 761 (50 and 100 mg/kg, p.o.) and an extract devoid of terpenes (CP 205; 100 mg/kg, p.o.) on the motor behavior of rats subjected to unilateral motor cortex aspiration indicated that the active constituents contributing to this effect of accelerating recovery from cortical damage are not terpenes [93,94]. Other results, in showing that repeated treatment of rats bearing entorhinal cortex lesions with EGb 761 can promote significant recovery of spatial learning in the Morris water maze, provide further support for a beneficial effect of the extract in traumatic brain injury [95].

Using Timm's silver-sulfide staining method, Barkats *et al.* [96] determined the effects of EGb 761 treatment on age-associated changes in the projection fields of hippocampal mossy fibers (MFs). Commencing when female mice were 15 months old, they received either EGb 761 (50 mg/kg/day, p.o.) chronically for 7 months or no treatment. EGb 761 treatment led to a significant increase in the projection field of intra- and infrapyramidal mossy fibers (iipMF) in the CA3 region of the hippocampus and to a significant decrease in the area of the stratum radiatum, as compared to control mice. As the MF system, which relays neural information from the entorhinal area of the dentate gyrus to Ammon's horn, appears to be sensitive to the effects of ageing [97], EGb 761 treatment could help to maintain this particular innervation of the hippocampus in ageing mice. EGb 761 might both protect the iipMF against age-related degeneration and stimulate compensatory processes of synaptic plasticity ("sprouting") that follow age-related destruction of these fibers. As MFs may also be particularly sensitive to the age-related excessive formation of ROS, certain constituents of EGb 761 possessing antioxidant properties could have been involved in these effects. Thus, the improvements in memory performance observed in EGb 761-treated animals [98] may be connected with antioxidant actions of the extract that oppose age-related modification of MFs. In this regard, it is noteworthy that elevatedglucocorticoid levels produce hippocampal dysfunction and correlate with individual deficits in spatial learning in aged rats, that persistent increases in cortisol have been correlated with memory impairment in elderly human subjects, and that aged humans with significantly prolonged cortisol elevations show reduced hippocampal volume and deficits in hippocampus-dependent learning and memory tasks [99].

Collectively, the results discussed in this section have revealed that repeated systemic treatment with EGb 761 can influence learning and memory processes and exert beneficial effects on recovery of function after brain injury. Such effects of the extract, which may involve mechanisms associated with synaptic plasticity, are useful in explaining certain improvements in memory and other cognitive functions that follow EGb 761 treatment in both humans and experimental animals. Thus, further support is gained for the continued use of EGb 761 in treating AD and other age-associated disorders that are characterized by neurodegeneration, dementia and other cognitive impairments.

"Anti-stress" Activity of EGb 761

Results obtained with a rat model of "learned helplessness" and several other behavioral tests

have revealed that EGb 761 possesses an "anti-stress" action that differs from those of conventional antidepressants and anxiolytics and which is not related to an impairment of memory for aversive stimulation [100,101]. More recently, this "anti-stress" action of EGb 761 has been examined in relation to learning ability (discrimination task) and circulating levels of stress hormones (catecholamines and corticosterone) in rats subjected to a stressful situation [102]. Young (4-month-old) and old (20-month-old) rats, maintained under water restriction, were trained to discriminate by lever-pressing to obtain a small amount of drinking water as a reward. Introduction of an auditory perturbation ("stress") during the discriminative phase of learning decreased the capacity and rate of acquisition in both young and old animals. Repeated treatment with EGb 761 (50 and 100 mg/kg/day, p.o.) suppressed this stress-induced disruption of learning, an effect that became statistically significant by the third day of learning (time of maximal acquisition rate) and

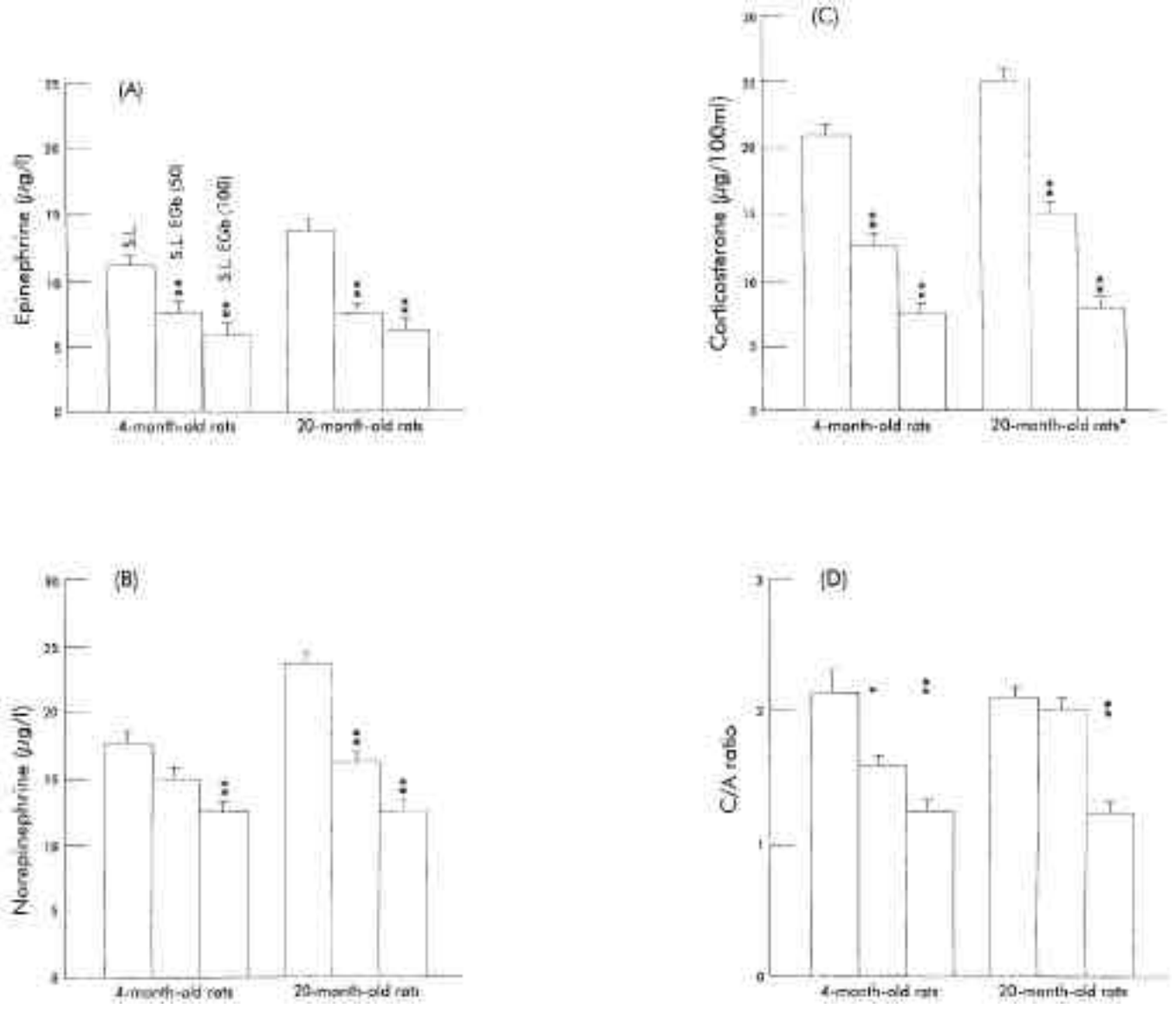


Fig. 3. Plasma concentrations of epinephrine, norepinephrine and corticosterone in young and old rats after the third day of discrimination learning, with and without auditory stress; effects of chronic (20-day) treatment with EGb 761. The code for treatment of the animals in each series of histograms is indicated in A (left) and is defined, from left to right, as follows: S,L = stressed, with learning; S,L, EGb (50) = stressed, with learning and treatment with EGb 761 (50 mg/kg p.o.); S,L, EGb (100) = stressed, with learning and treatment with EGb 761 (100 mg/kg, p.o.). [A, Epinephrine; B, norepinephrine; C, corticosterone; D, corticosterone/epinephrine ratio (C/A).] Means ± S.E.M.; 10 rats in all cases; * and ** indicate, respectively, $p \leq 0.05$ and $p \leq 0.01$ for comparisons among animals of the same age group (Student's t-test). (Reproduced with permission from Rapin *et al.* [102]).

which was more pronounced in the older animals. The deleterious effects of stress on learning ability were clearly reflected as increases in circulating concentrations of epinephrine and norepinephrine (NE) and with an even greater augmentation in circulating corticosterone in both young and old rats, and these changes were significantly reduced by 20 days of EGb 761 treatment in both young and old rats; see Fig. (**3**); see also [103].

A more recent study, involved rats that were subjected to three different types of stress [104]. Subjecting the animals to cold stress, restraint stress, and auditory stress (distributed randomly to eliminate habituation of the animals) for 15 days led to significant decreases in the endogenous content of NE in both the hippocampus and hypothalamus. The turnover rate of NE, examined after inhibition of its synthesis by α-methyl-p-tyrosine (50 mg/kg,i.p.), was significantly increased in these same brain regions. Treatment of the animals with EGb 761 (50 or 100 mg/kg/day p.o.) for 8 days did not modify the cerebral content or turnover of NE in unstressed animals, but significantly decreased in the effects of stress in stressed animals; i.e., treatment with the extract was associated with a less significant augmentation in NE turnover and with a smaller decrease in endogenous NE content. In contrast, iproniazid slowed the turnover of NE to the same extent in unstressed and stressed rats, an effect that was correlated with increases in the endogenous level of NE in the two brain structures examined. From these results, it was concluded that EGb 761 is an "anti-stress" agent that protects against the deleterious effects of stress by acting at the level of the central noradrenergic system, whereas iproniazid, by acting as a non-selective inhibitor of monoamine oxidase (MAO), modifies noradrenergic neurotransmission via an action that is associated with antidepressant activity but which cannot be considered to be "anti-stress".

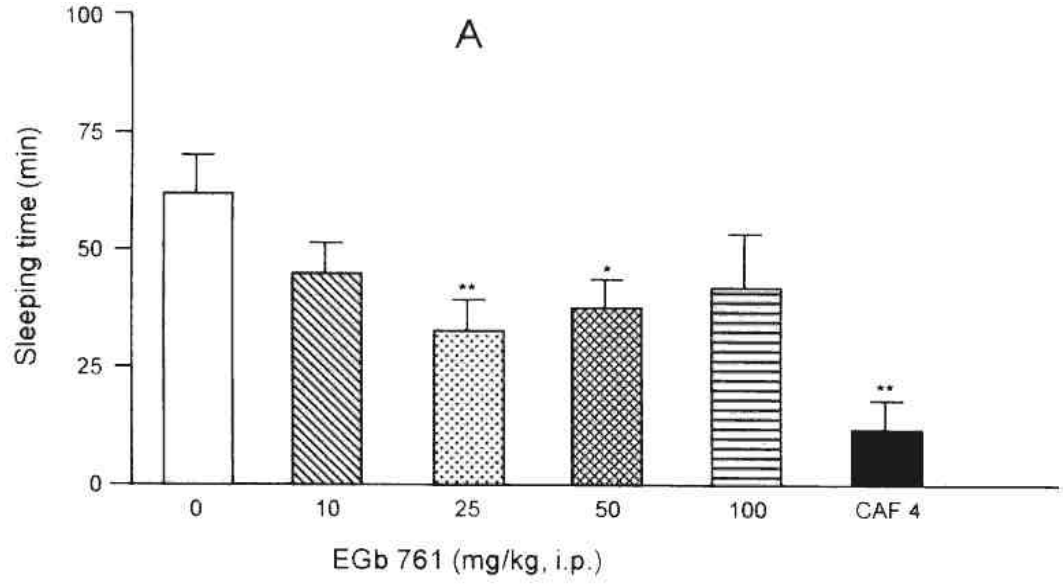
The "social interaction" test has also been used to define the mechanism(s) underlying the "anxiolytic-like" ("anti-stress") effect of EGb 761 [105]. The interactions of EGb 761 with diazepam (an anxiolytic "positive control" substance that acts as a full agonist at the $GABA_A$/benzodiazepine/Cl^- channel complex) and ethyl β-carboline-3-carboxylate (β-CCE; an anxiogenic substance and a partial inverse agonist at the benzodiazepine site of the $GABA_A$ receptor complex [106]) were examined. Pairs of "unfamiliar" male rats that had been subjected to the same treatment were placed in a novel test arena that was brightly illuminated, and the duration of social contact was observed over a 10-minute period. Single injections of EGb 761 (8-16 mg/kg, i.p.), given 30 minutes prior to testing, or repeated oral administration of EGb 761 (48 or 96 mg/kg/day) for 8 days, significantly decreased social contact. This finding, which could be defined as being "anxiogenic-like" and which implies an increase in "arousal" or "emotionality", supports the clinical use of EGb 761 in enhancing vigilance. In contrast, administration of a single injection of diazepam (1 mg/kg, i.p.), administered 30 minutes before commencing the test, to animals that had previously received repeated oral treatment with EGb 761 (96 mg/kg/day) increased social interaction to an extent greater than observed with diazepam alone. Other experiments showed that EGb 761 can neutralize the anxiogenic effect of β-CCE. The molecular target(s) underlying the interactions of EGb 761, diazepam and β-CCE remain unknown, but could involve a similar site or distinct sites of action at the level of the central $GABA_A$/benzodiazepine/Cl^- channel receptor complex. Activation of the benzodiazepine binding site of this receptor complex facilitates the action of GABA (an inhibitory neurotransmitter) of opening Cl^- channels, which would favor anxiolytic effects; see [107].

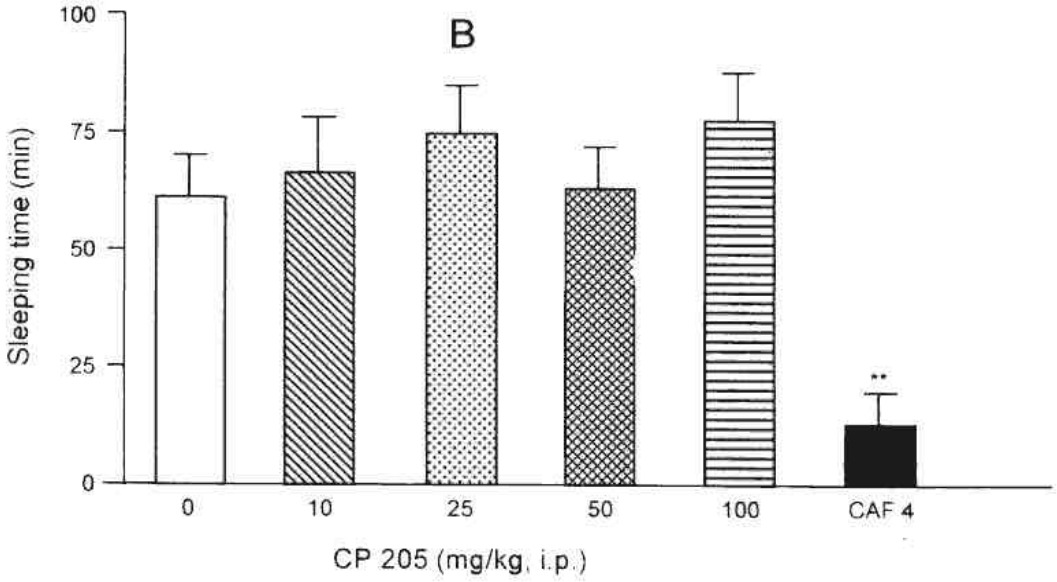
The very recent study of Brochet *et al.* [108] also involves central GABA receptors. Using a mouse model of barbital-induced narcosis, it was shown that single injections of EGb 761 (25 and 50 mg/kg, i.p.), given 60 minutes prior to sodium barbital, significantly shortened barbital-induced sleeping time, and that these same doses of CP 205 (Ginkgo extract devoid of terpene trilactones) were ineffective; see Fig. (**4**). Single injections of ginkgolide B (1 mg/kg, i.p.) and bilobalide (2 and 5 mg/kg, i.p.) also significantly shortened sleeping time, whereas ginkgolide A was ineffective; see Fig. (**4**). At the behavioral level, these potent effects of EGb 761, ginkgolide B and bilobalide resemble those of certain antidepressants, and therefore they are useful in explaining the "antidepressant-like" ("vigilance-enhancing") and "anti-stress" actions of EGb 761 that have been observed in clinical studies of both healthy subjects and patients with depressive episodes and/or dementia [3,21; see below].

In this latter study [108], barbital was selected as the barbiturate for testing since it is not metabolized by hepatic microsomal enzymes. Therefore, the actions of EGb 761 or its terpene trilactone constituents of shortening of barbital-induced sleeping time in this model can be considered to be direct effects on the CNS that are related to increases in "arousal" or "vigilance". At the molecular level, upon considering that barbiturates

act by facilitating Cl^- entry via $GABA_A$ receptor-associated Cl^- channels in the CNS and that this action can be opposed by picrotoxin [107], it was hypothesized that EGb 761 and its terpene trilactone constituents acted at a site situated on or near the Cl^- channel of the $GABA_A$ receptor complex. Activation of the picrotoxinin/t-butylbicyclophosphorothionate (TBPT) site, which is associated with a decrease in inhibitory GABA function [107], appears to be the primary molecular target site involved. This contention is strengthened by the recent finding that a 30-minute intraperitoneal pretreatment with EGb 761 enhanced the pro-convulsant effects of picrotoxin in mice [109].

Another study has indicated that EGb 761 and diazepam differentially influence the capacity of prednisolone to reversibly down-regulate hippocampal Type II glucocorticoid receptors, to reduce acquisition lag time in a conditioned avoidance response (pole-climbing test), and to decrease reaction time and increase the number of errors in a Skinner box [110]. In this model, repeated treatment of rats with EGb 761 (50 mg/kg/day, p.o.) for 14 or 21 days suppressed the down-regulation of glucocorticoid receptors ([^{3}H]dexamethasone binding) induced by prednisolone treatment (50 mg/kg/day, i.p. for 5-15 days) and normalized learning parameters in both





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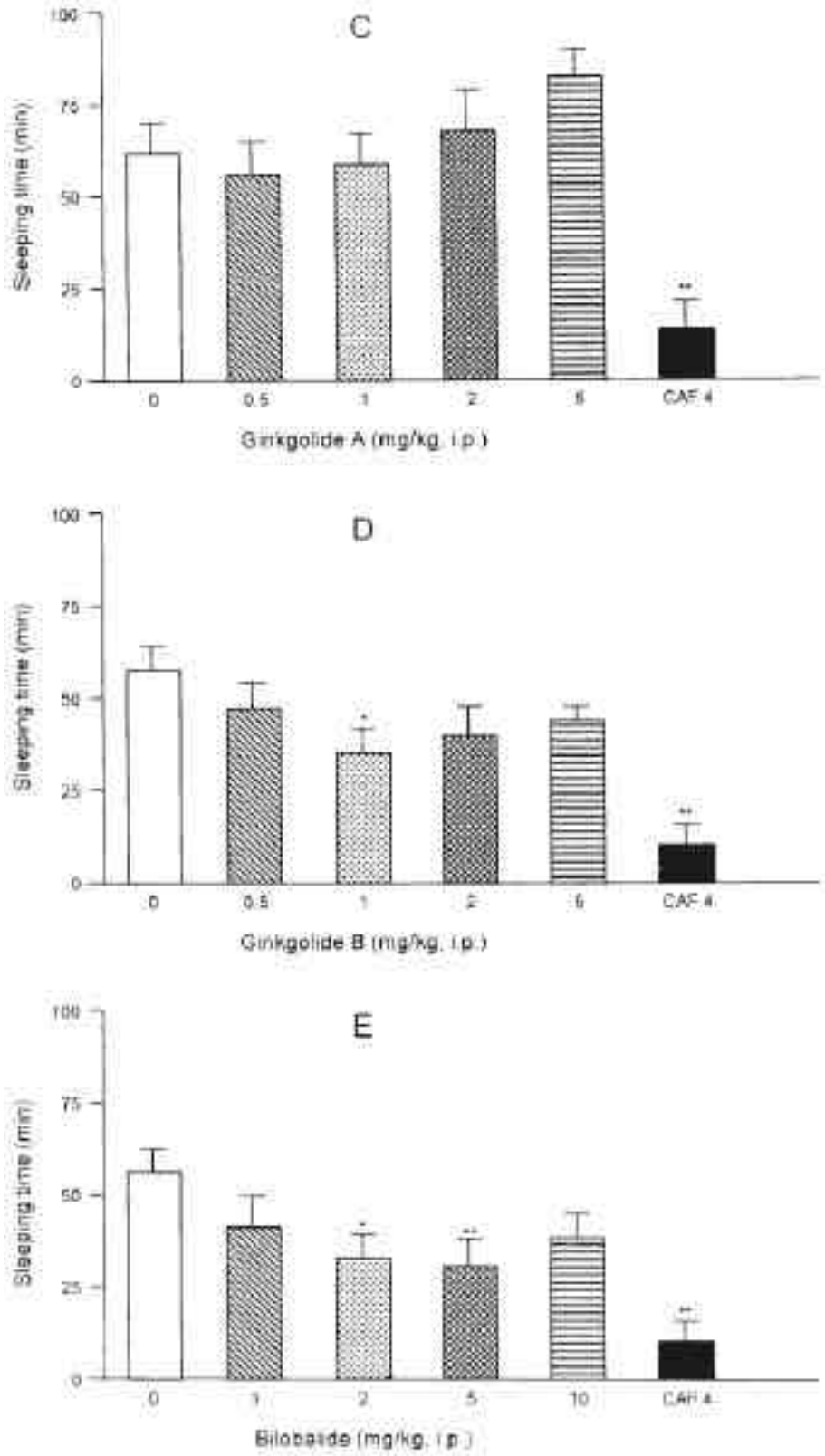

Fig. 4. Duration of barbital-induced narcosis ("sleeping time") in mice that received single intraperitoneal pretreatments with EGb 761, terpene trilactone-free extract (CP 205), terpene trilactones or caffeine. Means ± S.E.M.; 12 animals in all cases; * and ** indicate respectively $p < 0.05$ and $p < 0.01$ for comparisons between these values and the corresponding value for vehicle-treated controls. A, EGb 761; B, CP 205; C, ginkgolide A; D, ginkgolide B, E, bilobalide; CAF 4 = caffeine (4 mg/kg, i.p.). (Reproduced with permission from Brochet *et al.* [108]).

the avoidance and the discriminative behavioral tests, whereas repeated treatment with diazepam (10 mg/kg/day, p.o.) for 21 days further decreased [^{3}H]dexamethasone binding to glucocorticoid receptors and decreased learning parameters in both behavioral tests. It was contended that the deleterious effects of glucocorticoids on hippocampal glucocorticoid receptors and on cognitive performance can be suppressed by EGb 761 but not by the conventional anxiolytic drug, diazepam [110].

A more recent study has revealed that EGb 761 treatment can decrease the development of amphetamine-induced behavioral sensitization by an effect that also involves a suppression of the down-regulation of hippocampal type-II glucocorticoid receptors [111]. Pretreatment of rats with EGb 761 (50 or 100 mg/kg/day, p.o.), commencing 8 days before administration of D-amphetamine, reduced the behavioral sensitization induced by successive intraperitoneal injections of D-amphetamine (0.5 mg/kg/day, i.p.), as estimated from values for locomotor activity. EGb 761 treatment also prevented the decrease in density of [^{3}H]dexamethasone binding sites in the dentate gyrus and CA1 hippocampal regions of D-amphetamine-treated animals. These observations led to the suggestion that EGb 761, by reducing glucocorticoid levels, could modulate the activity of neuronal systems that are involved in the expression of behavioral sensitization. These findings are related to the previous result that repeated treatment of rats with EGb 761 decreases circulating levels of glucocorticoids under stress stimulation [65; see below], and furthermore they indicate that repeated treatment with EGb 761 can reduce the development of behavioral sensitization induced by successive administrations of D-amphetamine. Thus, it was hypothesized that EGb 761 treatments can oppose the effect of D-amphetamine injections which lead to excessive corticosterone secretion and to a persistent down-regulation of hippocampal glucocorticoid receptors, thereby disrupting the negative feedback system within the hypothalamo-hypophysial-adrenal axis and resulting in further increases in glucocorticoid secretion. Such an effect of EGb 761 of restoring regulatory activity within the hypothalamo-hypophysial-adrenal axis could involve a restriction of changes in dopaminergic neurones of the ventral tegmental area of the brainstem which are involved in mediating the increased locomotor activity that follows administration of D-amphetamine during the development of behavioral sensitization. These results are of interest since activation of the central dopaminergic system is generally involved in mechanisms underlying drug abuse.

The effects of EGb 761 (50, 100 and 150 mg/kg/day, p.o.), administered for five days per week during 14 days, have also been examined on heat stress-induced brain damage [112]. On the 15th day of experimentation, subjecting untreated animals to four hours of heat-stress (38^o C) elicited profound hyperthermia (41.89 ± 0.23^o C), behavioral salivation and prostration, and marked increases in blood-brain-barrier (BBB) permeability and brain water content, as compared to normal animals. Heat-stressed animals that had been treated with EGb 761 (100 and 150 mg/kg/day) showed only moderate hyperthermia (39.64 ± 0.45^o C), less marked behavioral anomalies, and only slight changes in BBB permeability and brain water content. Thus, EGb 761 treatment can counteract cellular changes in the brain that follow heat-stress.

As mentioned above, it is well known that excessive NO production can lead to deleterious effects [43,44]. Heat stress is one of the conditions that can lead to excess NO, which in turn causes edema and cellular damage by a mechanism involving an up-regulation of neuronal NO-synthase (nNOS). Sharma *et al.* [113] have shown that exposure of rats to four hours of heat stress (38^o C) led to a marked up-regulation of nNOS in brain regions that exhibited deterioration of the BBB, brain edema and cell damage. Pretreatment with EGb 761 (50 mg/kg/day, p.o.) for 5 days significantly attenuated nNOS expression, BBB disruption and brain injury. EGb 761 reduced the deterioration in BBB permeability in those brain regions that were associated with NOS up-regulation. It was suggested that EGb 761 exerted this neuroprotective action against heat stress by reducing the formation of ROS, resulting in decreased oxidative stress and reduced NOS expression.

Cold-stress (exposure to 4-5^o C) has also ben used to examine the neuroprotective effect of EGb 761. Bolanos-Jiménez *et al.* [114] assessed the capacity of EGb 761 to protect against cold-stress-induced changes in the function of hippocampal 5-HT_{1A} receptors in rats subjected to isolation rearing (individual housing). Inhibition of forskolin-stimulated adenyl cyclase activity by 8-OH-DPAT (the prototype 5-HT_{1A} receptor full agonist) was used to determine the functional activity of hippocampal 5-HT_{1A} receptors. Exposure of elderly (18-month-old) male rats to cold-stress for 5 days significantly decreased the inhibitory effect of 8-OH-DPAT on forskolin-stimulated adenyl cyclase

activity, indicating decreased functional efficacy, or a "desensitization" of 5-HT_{1A} receptors. As neither the affinity nor the density of hippocampal 8-OH-DPAT binding sites was affected, this cold-stress-induced desensitization of 5-HT_{1A} receptors was probably due to a modification of their coupling mechanism(s) to adenyl cyclase. Pretreatment of the animals with EGb 761 (50 mg/kg/day, p.o.) for 14 days prevented this cold-stress-induced desensitization of 5-HT_{1A} receptors, but did not appear to displace the specific binding of [^{3}H]-8-OH-DPAT to 5-HT_{1A} sites. Thus, EGb 761 treatment may restore the capacity of aged rats to adapt to a stressor via a mechanism that may not involve a direct interaction with 5-HT_{1A} receptors.

Other experiments conducted by Amri *et al.* [65] have shown that ginkgolide constituents of EGb 761 may mediate its "anti-stress" effects. Repeated treatment of rats for 8 days with EGb 761 (10-100 mg/kg/day, p.o.) or with its active constituents, ginkgolides A and B (2 mg/kg/day, i.p.; a dose which represents the approximate combined percentage of these two ginkgolides in 100 mg/kg of EGb 761), specifically reduced the ligand binding capacity, protein (determined by immunoblot analyses) and mRNA expression (determined by Northern blot analyses) of the peripheral benzodiazepine receptor (PBR) of adrenocortical mitochondria. The rat PBR appears to consist of two sites that may overlap (but there may also be more proteins to form sites for ligand binding). These are the 18-kDa isoquinoline site which is identified by the binding of PK 11195 [1-(2-chlorophenyl)-N-methyl-(1-methyl-propyl)-3-isoquinolinecarboxamide] ($K_D \simeq$ 1-5 nM), and the 34-kDa benzodiazepine site which is identified by the binding of Ro 5-4864 (4'-chlorodiazepam) and which may be functionally coupled to an anion channel. This receptor complex, usually localized to the mitochondrial outer membrane, is a key element in the regulation of cholesterol transport.

The ginkgolide-induced decrease in PBR expression led to decreases in corticosteroid synthesis and in circulating glucocorticoid levels, causing increased adrenocorticotrophic hormone (ACTH) release which in turn induced the expression of the steroidogenic acute regulatory protein (StAR). Since ginkgolides reduced adrenal PBR expression and corticosterone synthesis, despite the presence of high levels of StAR, these data demonstrated that PBR is essential for normal adrenal function. In these experiments, it should be noted that under the conditions employed the untreated ("control") rats had elevated circulating levels of corticosterone representative of stress, and that treatment with the ginkgolides restored the basal level of corticosterone. These results [65], in showing that the in vivo modification of PBR expression by EGb 761 or its ginkgolide constituents can modify circulating glucocorticoid levels, provide evidence that the "anti-stress" and, therefore, the neuroprotective effects of EGb 761 are due, at least in part, to its effect on glucocorticoid biosynthesis. More generally, these results indicate that EGb 761 may oppose various disease states that involve glucocorticoid excess, including neurotoxicity, neuroendangerment and immunosuppression. Glucocorticoid neurotoxicity has been associated with neurological diseases related to hippocampal ageing [e.g., 99, 115]. These results also substantiate the view that the mechanism(s) underlying the "anti-stress" action of EGb 761 differs from those mediating the actions of classical anxiolytics and antidepressants.

The data of Amri *et al.* [65] showed that repeated treatment of adult rats for 8 days with EGb 761 caused a dose-dependent decrease in circulating corticosterone concentration, a 50% decrease being observed with 100 mg/kg/day of the extract. EGb 761 or the ginkgolides had no effect when the rats were sacrificed acutely only three hours after treatment. Although repeated treatments with ginkgolides A and B caused significant decreases in serum corticosterone levels, bilobalide had no effect; see Fig. (**5**). Also, as serum aldosterone and testosterone levels were not modified, these effects appeared to be specific to the glucocorticoid-producing fasciculatis-reticularis cells of the adrenal cortex; see Fig. (**5**). As ginkgolides A and B, but not the total EGb 761 preparation, caused the expected increases in plasma ACTH (Fig. (**5**)), the total extract contains other active constituents that may inhibit the release of ACTH from the pituitary gland or CRF release from the hypothalamus [116]; see Fig. (**6**). The presence of this inhibitor(s) supports the use of the total EGb 761 preparation for therapy since it should protect against the deleterious effects of continuously elevated ACTH levels.

Treatments with EGb 761, ginkgolide A or ginkgolide B markedly decreased the number of PK 11195 binding sites in adrenal mitochondria, but did not affect such binding in kidney mitochondria. These results, taken together with other results, indicate that EGb 761 and its ginkgolide constituents preferentially reduce the expression of the 18-kDa isoquinoline-binding protein (a cholesterol channel/transporter protein in rat adrenal cortex [see also 117].

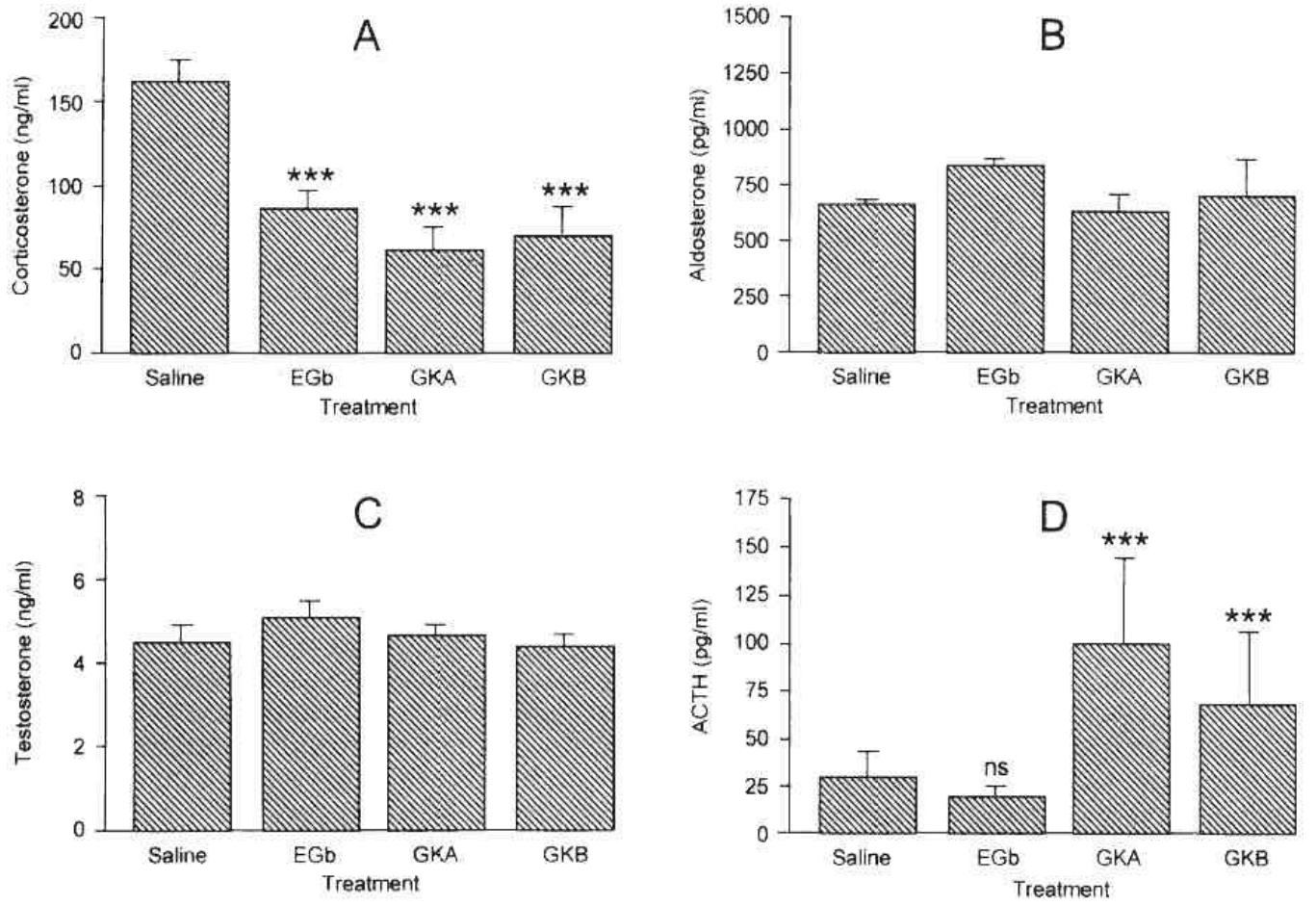

Fig. 5. In vivo effects of EGb 761, ginkgolide A (GKA) and ginkgolide B (GKB) on circulating levels of steroid hormones and ACTH. Adult Sprague-Dawley rats were treated daily with either EGb 761 (100 mg/kg, p.o.), ginkgolides (2 mg/kg, i.p.) or physiological saline for 8 days. Animals were sacrificed at Day 9 and serum or plasma was collected. Serum corticosterone (A), aldosterone (B) and testosterone (C) were measured by radioimmunoassay. Plasma ACTH levels (D) were also determined by radioimmunoassay upon chromatographic separation of the peptide. Results represent the means ± S.E.M. (n = 12 to 16) from 3 independent experiments; ns, not significantly different from control; ***, $p < 0.001$ compared to control. (Reproduced with permission from Amri, H., Ogwuegbu, S. O., Boujrad, N., Drieu, K. and Papadopoulos, V. [Ref. 65] In vivo regulation of the peripheral-type benzodiazepine receptor and glucocorticoid synthesis by the Ginkgo biloba extract EGb 761 and isolated ginkgolides. Endocrinology, 137, pages 5707-5718, with permission from the Endocrine Society).

These findings were confirmed by immunocytochemical studies using antisera specific for the 18-kDa protein which showed dramatic decreases in adrenal 18-kDa PBR protein expression after repeated treatment with EGb 761 or ginkgolides A or B, and by densitometric immunoblot analyses of mitochondrial extracts which revealed that the treatments reduced PBR mRNA expression, implying their direct effect on PBR mRNA stability or DNA transcription. A genuine down-regulation (reduced expression) of the PBR is evident from the findings that levels of mRNA, protein and receptor binding of this receptor were reduced by repeated treatment but not by acute treatment with EGb 761 or the ginkgolides.

The effects of EGb 761 on serum corticosterone levels and adrenal PBR expression were reversible, both parameters returning to normal in a time-dependent manner upon terminating the experiment, findings which further supported a cause-effect relationship between the effect of EGb 761 on PBR expression and steroid synthesis. Further experiments with an in vitro system, ACTH-responsive human adrenocortical carcinoma cell line NCI-H-295R, showed that addition of ginkgolide B to the medium inhibited dose-dependently both basal and hormone- (cyclic-AMP-) stimulated cortisol production at concentrations similar to that used in vivo. These results indicated that ginkgolide B acts directly on adrenal cortical cells and provided evidence that EGb 761 and ginkgolides could exert similar effects in humans.

This effect of EGb 761 and ginkgolides may be generalized to include other receptors or other proteins. In this regard, Amri *et al.* [118] have conducted experiments with adrenocortical cells that were isolated from rats treated with EGb 761 or ginkgolide B and cultured for 2 and 12 days. The effects of ACTH on normal and metabolically labelled cells were examined. Ex vivo treatment with EGb 761 and ginkgolide B resulted, respectively, in 50% and 80% reductions of ACTH-stimulated corticosterone production by adrenocortical cells cultured for 2 days, as compared with cells isolated from saline-treated rats. ACTH induced the synthesis of a protein identified as StAR to the same extent in cells from control and EGb 761- or ginkgolide B-treated animals. Treatment with EGb 761 and ginkgolide B specifically altered the synthesis of seven proteins, including inhibition of synthesis of a 17-kDa protein identified as PBR. After 12 days in culture, ACTH-stimulated adrenocortical steroid synthesis was maintained, and it was identical among the cells isolated from animals treated with ginkgolide B or saline. Under the same conditions, the expression of PBR was recovered, whereas no effect of ACTH was observed on the synthesis of StAR or other proteins. A comparative analysis of the effects of ginkgolide B and EGb 761 on adrenocortical steroidogenesis and protein synthesis identified, in addition to the 17-kDa PBR, target proteins of 32 kDa and 40 kDa as potential mediators of the effect of EGb 761 and ginkgolide B on ACTH-stimulated glucocorticoid synthesis. These results validated and extended previous in vivo findings on the effects of EGb 761 and ginkgolide B on ACTH-stimulated adrenocortical steroidogenesis in demonstrating the specificity and reversibility of EGb 761 and ginkgolide B treatments and the obligatory role of PBR in hormone-regulated steroidogenesis.

These findings [65,117,118] indicate that both the "anti-stress" and neuroprotective effects of EGb 761 are due, at least in part, to an action of two of its ginkgolide constituents, and that peripheral glucocorticoid biosynthesis serves as their molecular target. They bear an obvious relationship to recent findings which imply that basal cortisol elevation, such as that which occurs during human ageing, may cause hippocampal atrophy and impair hippocampus-dependent learning and memory, and that altered cortisol responsiveness to acute and/or chronic stress, with its detrimental effects on memory, could be an important factor for explaining the genesis of memory deficits in aged populations [99,119-121].

Collectively, the results discussed in this section substantiate the view that the mechanism(s) and molecular targets underlying the "anti-stress" action of EGb 761 differ from those mediating the actions of classical anxiolytics and antidepressants.

CLINICAL STUDIES

Ever since its introduction into clinical medicine, EGb 761 has been used primarily to treat CNS disturbances involving impairment of cognition, vigilance and mood, and associated neurosensory symptoms (e.g., memory loss, vertigo, tinnitus). Clinical studies conducted during the past two decades have revealed that EGb 761 is efficacious in treating a wide range of symptoms associated with both early cognitive decline and more severe types of senile dementias of primary degenerative (e.g., AD), vascular and mixed origins [3,21-24,27,122-124]. In such cases, it appears that EGb 761 improves CBF and brain energy metabolism and protects against the deleterious effects of excessive formation of ROS, cerebral ischaemia and cerebral edema [3, 125]. Such beneficial effects of EGb 761 generally become evident 4-8 weeks after commencing its oral administration, and they may become more pronounced after 3-6 months.

In attempts to explain the clinical actions of EGb 761, one must consider that it has several different mechanisms of action involving diverse cellular and subcellular targets which, as mentioned above, have been demonstrated using various in vitro and in vivo systems. In particular, EGb 761 has "cognition-activating" and "anti-stress" properties, improves CBF and microcirculation, counteracts hypoxia, improves blood rheology and tissue metabolism, and reduces capillary permeability. Antioxidant/free radical-scavenging and anti-lipoperoxidative activities of the extract, its ability to preserve membrane function, its action of reversing age-related losses in neurotransmitter receptor density, and its action of reducing circulating levels of glucocorticoids may also be significantly involved in the observed clinical effects.

Among the prime clinical indications for EGb 761, "degenerative dementia" may be defined as the AD-type, "cerebrovascular dementia" is considered to be associated mainly with multiple brain infarcts, and a "mixed" form consists of both types of pathology. The major symptoms of all three types are similar (e.g., memory disturbances, reduced vigilance, loss of concentration, instability of mood, dizziness, headache, tinnitus and, in certain

cases, depression). All three types of dementia are associated with chronic cerebral insufficiency. In addition, the brains of patients with AD are characterized by excessive deposits of "amyloid" protein. It is noteworthy that the most consistently observed electroencephalographic (EEG) characteristics of persons with dementia are decreases in the relative power frequencies of the alpha (physiological) and beta-1 wave bands and increases in the relative power frequencies of the slow theta and delta wave bands [126].

Vascular and degenerative forms of dementia are generally differentiated using the ischaemic scale of Hachinski *et al.* [127]. Other rating scales, some of which have pronounced subjective components, are used to assess the severity of dementia and the effects of therapy. However, it remains extremely difficult, even with double-blind trials, to assess precisely and objectively the results of treatments aimed at improving the intellectual performance of elderly patients. For this reason, strict guidelines have been used in the more recent trials aimed at determining the clinical efficacy of EGb 761 as an anti-dementia treatment [128,129]. It is maintained that studies conducted in accord with these guidelines may yield pharmacologic therapies for cognitive decline and for the behavioral problems associated with AD and other forms of dementia, thereby improving quality of life.

This section deals with clinical pharmacology and trials of EGb 761-containing products as symptomatic treatment for CNS disturbances that generally occur in elderly patients and which include the dementias associated with cerebrovascular insufficiency and/or progressive neurodegeneration.

Studies on Cerebral Blood Flow

Early trials in patients and human volunteers implied that regional and global CBF, as well as cerebral glucose and oxygen consumption, can be augmented by administration of EGb 761 [see 3,21]. In one of these open studies, treatment of 19 patients afflicted with neurologic ischaemic syndrome of brain areas supplied by the carotid and vertebrobasilar arteries with EGb 761 (160 mg/day for 2 months) increased cerebral perfusion significantly, such that it approached the normal value [130]. Significant increases in glucose and oxygen consumption and a significant improvement in behavioral performance were also noted in these EGb 761-treated patients. Such effects are required of agents that are used to treat dementia and related disorders.

Chronic Cerebrovascular Insufficiency

"Chronic cerebrovascular insufficiency", also specified as "chronic cerebral insufficiency", increases as a function of increasing age of the subject, likely due to advancing atherosclerosis and its many complications. This type of disorder is characterized by many psychopathological symptoms, including impairments of intellectual, cognitive and emotional capacities, tinnitus, headaches, vertigo, depression and anxiety states. Early studies have shown beneficial effects of Ginkgo extracts (in some cases, authentic EGb 761) in patients suffering from cerebrovascular insufficiency and related conditions, and more recent studies have confirmed and extended these results using EGb 761 [see 3,21]. Some of these studies are briefly outlined below.

The placebo-controlled, randomized double-blind study of Eckmann and Schlag [131] was aimed at testing the efficacy of EGb 761 (120 mg/day) in 50 patients with cerebrovascular insufficiency. It was found that pathological symptoms decreased much earlier in EGb 761-treated patients (median of 12 days) than in placebo-treated controls, and after 30 days of treatment the symptoms that were examined were eliminated in 91-100% of EGb 761-treated patients, whereas this occurred in only 43-57% of placebo-treated patients. Efficacy of EGb 761 was also apparent upon comparing neurological and psychological findings, and no undesirable side-effects or significant changes in clinical laboratory tests were observed.

The study of Vorberg [132] dealt with 112 outpatients (mean age 70.5 years) afflicted with chronic cerebral insufficiency. Treatment with EGb 761 (120 mg/day) in this open one-year trial led to statistically significant regression of vertigo, headache and tinnitus, as well as beneficial effects on short-term memory, vigilance and mood (Crichton Scale). EGb 761-treated patients continued to improve during the course of this study, and no significant cardiovascular changes or adverse side-effects were noted.

The reference-controlled, double-blind trial conducted by Brage [133] involved 42 patients (aged 60-80 years) afflicted with "mild-to-moderate" chronic cerebral insufficiency (Crichton Scale). Half of the patients received EGb 761 (120 mg/day for 12 weeks) and the other half were treated with dihydroergotoxine mesylate (4.5 mg/day). Results based on analyses of cognitive function, physical alterations and everyday

activities (Crichton) showed clinical efficacy of EGb 761, as assessed by a physician and by the patients. Treatments with EGb 761 and dihydroergotoxine mesylate were equivalent in efficacy at the single doses that were compared [see also 134].

The study of Taillandier *et al.* [135] was performed to test the efficacy of EGb 761 as therapy for elderly patients with chronic cerebral disorders. In this multi-centric, double-blind and placebo-controlled study of 166 patients (mean age 82 years), treatment consisted of EGb 761 (160 mg/day) or placebo, and examinations were carried out at 3, 6, 9 and 12 months. By the third month of treatment, scores obtained with a specially designed Geriatric Clinical Evaluation Scale [GCES; the French version of the Sandoz Clinical Assessment-Geriatric test (SCAG)] showed a statistically significant difference between EGb 761 and placebo treatments. Thus, an efficacy of EGb 761 can be inferred at the level of producing a desirable change in everyday behavior and social integration.

The study of Halama *et al.* [136] was randomized and double-blind and included 40 outpatients (55-80 years of age) diagnosed with "mild-to-moderate" cerebrovascular insufficiency (Hachinski score >7; Crichton score 1-3). [The Hachinski ischaemic scale was used to differentiate vascular and primary dementia, and the Crichton scale was used to assess the degree of severity of symptoms.] Upon comparing 20 patients treated with EGb 761 (120 mg/day for 12 weeks) and 20 placebo-treated controls, a highly significant beneficial effect of EGb 761 treatment was found on the psychopathological level, particularly on disturbances of short-term memory, mental alertness and dizziness. Psychometric evaluation with the "Syndrom Kurz Test" (SKT) showed that the total score in EGb 761-treated patients decreased from 7 to 3.5, whereas only slight changes occurred in the placebo group. This study further supports the efficacy of EGb 761 as symptomatic treatment for patients with "mild-to-moderate" cerebrovascular insufficiency. Other earlier clinical studies are not as convincing as this study [136], partly because of inadequate experimental designs and the use of statistical methods which did not comply with present-day requirements.

The double-blind, randomized, placebo-controlled trial conducted by Wesnes *et al.* [137] involved elderly patients (mean age 71 years), 27 of whom were treated with EGb 761 (120 mg/day) and 27 others with placebo. These patients suffered from mild idiopathic cognitive impairment that was likely of non-vascular origin (Crichton score >14; Hachinski score <5). Before and after the study, cognitive efficacy was assessed by a pre-defined battery of tests and "quality of life" was determined using a behavioral questionnaire. EGb 761 was significantly superior to placebo for the combined scores of the test battery at weeks 8 and 12 and for rapidity of performance at week 4 (see also below).

The multi-center, randomized, double-blind placebo-controlled study of Gräβel [138] was conducted to examine the effect of EGb 761 on basic parameters of mental performance in 72 outpatients (mean age, 63.8 years) afflicted with cerebral insufficiency. Patients received either EGb 761 (160 mg/day) or placebo (36 patients in each group). Fifty-three of the patients were accepted for the final evaluation; dropouts were not connected with the medication. Application of computer-assisted, psychometric examinations of short-term memory and basic learning rate revealed statistically significant improvements in short-term memory after six weeks and in learning rate after 24 weeks in the EGb 761-treated group, whereas such changes were not found in the placebo group. These and other tests indicated that basic functions involved in conscious information-processing were improved by EGb 761 treatment.

Collectively, these studies have revealed that EGb 761 treatment has beneficial effects in patients afflicted with chronic cerebrovascular insufficiency and that these effects are associated with minimal or negligible adverse side effects and no modification of clinical hematological or biochemical parameters (see also results of meta-analyses, discussed below).

"Vigilance-Enhancing" Action of EGb 761

As already mentioned above, a major effect of EGb 761 treatment is increased vigilance (alertness or awareness), an effect that is evident in EEG recordings of patients who receive the extract. Numerous clinical studies of patients suffering from disturbances of cerebral function and EEG signs of impaired vigilance have shown that EGb 761 treatment decreases the proportion of low frequencies and increases the alpha-portion of the total power spectrum. This results in a distinct decrease in theta/alpha ratio which denotes increased vigilance [see 3,21]. Several of the studies which have revealed that EGb 761 significantly modifies the quantitative EEG in both healthy volunteers and in patients suffering from various cerebral disorders are mentioned below.

The randomized, double-blind, placebo-controlled trial of Hofferberth [139] was focussed on examining the effects of EGb 761 (120 mg/day) or placebo on neurophysiological and psychometric parameters in 36 patients (mean age 63.3 years) with "cerebro-organic syndrome". Considering results obtained with various evaluation criteria, together with quantified EEG, saccade test, Vienna determination test, and "trail-making" test, significant differences between the two groups became apparent after four or eight weeks of therapy. The proportion of theta waves in the theta/alpha ratio of the EEG spectrum was significantly reduced in the EGb 761-treated group after eight weeks of therapy, as compared to placebo. The duration of rapid eye movements (saccade test) decreased with EGb 761 from 162 to 117 milliseconds, whereas no significant change occurred in the control group. The number of correct responses scored in the Vienna test increased with EGb 761 treatment, achieving significance by four weeks of therapy, and results in the trail-making test also differed significantly between EGb 761 and placebo after eight weeks. EGb 761 treatment was significantly superior to placebo for all parameters tested, indicating efficacy for the extract as therapy for cerebro-organic syndrome (also termed cerebral insufficiency, or cerebrovascular insufficiency; see above).

The study of Künkel [140] involved assessing the EEG effects of the total EGb 761 preparation, Fraction I (flavonoids of the total extract), or Fraction II (flavonoids-plus-ginkgolides), each taken at an oral dose of 240 mg/day. Results of this placebo-controlled, double-blind study with quadruple crossover design in 12 participants showed that these three preparations differed from one another with respect to their effects on the EEG. The major area of electrical activity was in the temporobasal region following administration of Fraction II and in the frontal region following Fraction I, and the total EGb 761 preparation produced changes in both of these regions. On this basis, Künkel [140] concluded that the total extract, the flavonoids, and the ginkgolides all appeared to be bioavailable to the brain. Although such a conclusion may be justified to some extent, upon considering certain results of basic studies [108] (see above), it should be noted that systemically administered substances can also influence the EEG via secondary and/or reflex actions originating in remote peripheral (extracerebral) target sites.

The study of Luthringer *et al.* [141] was performed to examine the effects of EGb 761 on various electrophysiological parameters (EEG mapping profile, EEG pharmacodynamic variables, dose and time effects, induced modifications of electrocerebral vigilance level and cognitive capacities). The mapping profile of EGb 761 obtained with 15 healthy volunteers resembled that of a nootropic drug, its effects being interpretable as an "enhancement of vigilance". EEG pharmacodynamic parameters of the drug were: central delay of action of 0.5 hour, peak action at about 2 hours, and duration of action at least as long as the kinetic duration.

As the vigilance-enhancing action of EGb 761 indicates that it is a "cognitive-activator", or "cognition-enhancer", the pilot bioequivalency study of Itil and Martorano [142] is of special interest. They used the "Quantitative Pharmaco-EEG" method (QPEEG) to compare the effects of three commercially available preparations of Ginkgo biloba leaf extract, Ginkgold®, which is equivalent to authentic EGb 761, Ginkgo Power® and Super Ginkgo® which are not equivalent to authentic EGb 761. CNS effects were compared in 12 healthy male volunteers (mean age 32.3 years) using a double-blind, crossover method, and computer EEG (CEEG)/dynamic brain mapping data werecollected before, and at 1 and 3 hours after oral administration of the Ginkgo products. At 1 hour after single-dose administration, Ginkgold® increased alpha activity (7.5-13 Hz) in all brain areas, Super Ginkgo® also increased alpha activity but to a lesser degree, and Ginkgo Power® elicited a minimal increase in alpha activity. Evaluation of the total frequency spectrum showed that Ginkgold® produced an increase in alpha activity and a decrease in slow and fast waves in the primary band of the CEEG analysis, changes that could be classified as those of a cognitive-activator. In this regard, it is noteworthy that drugs considered to be "proven" anti-dementia drugs, tacrine in the United States and the nootropics (e.g., piracetam) of Europe, like Ginkgold® (EGb 761), are all claimed to increase alpha activity and decrease slow waves in the EEG and to show cognitive-activating, "vigilance-enhancing" CEEG profiles; see also [143].

With further regard to a possible influence of EGb 761 on memory, Allain *et al.* [144] have used the "dual-coding test", a method that rates the memory coding of verbal material and images with respect to variable presentation times. In this randomized, double-blind study with crossover design, 18 men and women (ave. age 69.3 years) who were plagued by mild memory impairment received EGb 761 (320 or 600 mg) or placebo one hour before performing the dual-coding test. A 6-

day washout period separated each of the treatment periods. Results indicated that EGb 761 enhanced the rate of information processing (learning and storage) in the brains of these elderly patients.

Collectively, the results discussed above are of considerable significance with respect to defining the therapeutic efficacy of EGb 761. The "vigilance-enhancing" action of EGb 761 signifies a beneficial influence on cerebral circulation and metabolism and indicates that the extract has properties resembling those of known nootropic agents. These findings support the use of EGb 761-containing products in treating AD and other types of dementia.

EGb 761 and Primary Degenerative Dementia (Alzheimer's Disease)

Four clinical studies of EGb 761 deserve special attention since they provide support for the safety and therapeutic efficacy of the extract in "mild-to-moderate" forms of primary degenerative AD-type dementia, defined as subjects having a score <7 on the Hachinski scale [126,145-147].

The trial of Weitbrecht and Jansen [145] compared the effects of EGb 761 (120 mg/day) with both placebo and ergot alkaloids (5.94 mg/day) over a three-month period. Sixty patients (ave. age, 72.4 years) suffering from "mild-to-moderate" primary degenerative dementia (Hachinski scores <7) were examined in this randomized, double-blind placebo-controlled study that was also open and reference-controlled. Twenty patients served as placebo controls, 20 were treated with EGb 761 and 20 others were treated with ergot alkaloids. Evaluations were based on the psychopathological level (patient's own assessment; physician's global assessment; SCAG), on the psychometric level (Wechsler digit symbol substitution test; digit span test), on the neurophysiological/ dynamic functional level (flicker fusion frequency; reaction time) and on the behavioral level (Crichton Scale). The EGb 761-treated group showed statistically significant improvement both in psychometric tests and in scores on clinical scales after four weeks of treatment. With EGb 761 treatment, the Crichton Scale showed an improvement of 23.5% as compared to initial values, significant with respect to before/after differences in the placebo group at weeks 4, 8 and 12, and the SCAG total score was significantly improved by 33% with respect to initial values. The physician's evaluation, psychometric test results and both flicker-fusion frequency and reaction time also improved significantly with EGb 761 treatment, as compared to placebo. Patients treated with ergot alkaloids were also significantly improved, as compared with placebo, and the effect of this reference substance did not differ significantly from that of EGb 761 during any of the treatment phases.

The study of Hofferberth [126] assessed EGb 761 treatment in 40 patients with probable "mild-to-moderate" senile dementia of the AD-type. This was a randomized, double-blind study with parallel group comparison (EGb 761, 240 mg/day, 21 patients vs matching placebo, 19 patients). Evaluations of the patients at baseline and at 1, 2 and 3 months using a test battery that included the total score on the SKT as the prime target parameter, as well as the SCAG, choice reaction time, saccadic eye movements and EEG analysis as secondary parameters, indicated that all parameters, except for the SCAG, showed highly significant improvement (compared to placebo) after only one month of EGb 761 treatment. After 1, 2 and 3 months of therapy with EGb 761, the improvement in memory and attention (SKT test) was highly significant, and the theta/alpha quotient of the EEG was decreased, indicating enhanced vigilance (see also above). The highly significant difference in SKT results showed that EGb 761 treatment opposed cognitive deficits, adding support to the view that EGb 761 is useful in treating patients with "mild-to-moderate" degenerative dementia of the AD-type.

The study of Kanowski *et al.* [146] was prospective, randomized, double-blind, placebo-controlled and multi-centric. The efficacy of EGb 761 (240 mg/day) was assessed in 216 outpatients (>55 years of age) with "mild-to-moderate" presenile and senile primary degenerative dementia of the AD-type or multi-infarct dementia. Diagnosis was based on the Diagnostic and Statistical Manual for Mental Disorders (DSM-III-R criteria) in this 24-week trial. In accord with the recommended evaluation approach for proving efficacy of nootropic and/or anti-dementia drugs [128,148], three primary variables were selected: Clinical Global Impressions (CGI; Item 2) for psychopathological assessment, SKT for assessment of attention and memory, and Nuremberg Age Observation Scale (NAB) for behavioral assessment of activities of daily life. Data from 156 patients who completed the study (79 with EGb 761; 77 with placebo) were used to determine efficacy. After 24 weeks, responder rates in the single primary variables CGI and SKT were significantly higher with EGb 761 than with placebo. CGI showed improvement termed "better"

or "much better" for 32% of EGb 761-treated patients, as compared to 17% in the placebo group ($p < 0.05$), SKT showed that 38% of EGb 761-treated patients improved by at least 4 points as compared to 18% in the placebo group ($p < 0.005$) and the NAB showed an improvement of at least 2 points in 33% of the EGb 761-treated patients as compared with 23% in the placebo group (N.S.). The frequency of therapy responders in both treatment groups differed significantly in favor of EGb 761 treatment, and an "Intent-to-Treat (ITT)" analysis of 205 patients yielded similar results. These results confirmed the clinical efficacy of EGb 761 as therapy for "mild-to-moderate" dementia of the AD-type and multi-infarct dementia, and a subgroup analysis showed no difference in efficacy between these two types of dementia.

Most recently, Le Bars *et al.* [147] have conducted a placebo-controlled, double-blind, parallel-group, randomized trial to assess the efficacy and safety of EGb 761 (120 mg/day) in "mild-to-severely" impaired outpatients with AD or multi-infarct dementia. Primary outcome measures were: Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog), Geriatric Evaluation by Relative's Rating Instrument (GERRI) and Clinical Global Impression of Change (CGIC). Of the 327 patients enrolled in this study, 309 were included in an ITT analysis, and 202 provided data that could be evaluated in a 52-week endpoint analysis. Considering the ITT analysis, the comparison between EGb 761 and placebo showed statistically significant mean treatment differences favorable to EGb 761 on the ADAS-Cog and on the GERRI. Upon considering the AD population separately, the subgroup receiving EGb 761 showed an overall improvement on the ADAS-Cog and on the GERRI in the ITT and 52-week-endpoint analyses, whereas the placebo-treated group worsened, leading to a favorable treatment effect for EGb 761. Among the patients in the AD group receiving EGb 761, 53% achieved at least a 2-point and 29% achieved at least a 4-point improvement, compared to respective values of 28% and 13% with placebo ($p = 0.006$). On the GERRI, 39% were considered improved with EGb 761, compared to 20% with placebo ($p = 0.006$). When the moderately to severely impaired AD patients were analyzed individually, the treatment effect favoring EGb 761 was still observed on the ADAS-Cog and on the GERRI. This study revealed that EGb 761 was safe and that it could stabilize, and in a substantial number of cases, improve the cognitive performance and social functioning of AD and multi-infarct dementia patients for periods of six months to one year.

Meta-Analyses of EGb 761 Treatment

Five meta-analyses of therapy with EGb 761 and/or other Ginkgo biloba leaf extracts that have been published during the past decade will be briefly discussed below [22-24,27,124,149].

The meta-analysis of Weiss and Kallischnigg [149] was performed with data obtained in double-blind placebo-controlled trials of the therapeutic efficacy of EGb 761 for the indication disturbed cerebral performance, which includes cerebral or cerebrovascular insufficiency, various dementias, and other age-related cerebral disorders. Assessment of 17 studies (data available up to Feb. 1, 1988) from a biometric perspective indicated that five studies could be ignored and that two other studies showed neither significant nor relevant contributions regarding therapeutic efficacy of EGb 761. Of the 12 studies considered to be acceptable, 10 "confirmatory" as opposed to two "non-confirmatory" studies were found upon considering comparisons with one other, an assessment ratio (10:2) which clearly validated the therapeutic efficacy of EGb 761.

The meta-analysis of Kleijnen and Knipschild [22,23] involved 40 clinical trials of Ginkgo extracts for the indication of cerebral insufficiency (data acquired up to 1991). Leaf extracts of Ginkgo biloba, primarily EGb 761-containing products (Tanakan®, Tebonin® and Rökan®), but also including LI 1370 (Kaveri®; not identical to EGb 761) were employed in these trials. It was concluded that eight of the 40 trials, generally conducted for at least 4-8 weeks with 120 mg/day of either an EGb 761 product [135,137,150-155], were well performed and of acceptable quality, and that seven of these "acceptable" trials (total of 434 patients; ave. age, 50-82 years) showed effects of EGb 761 that were superior to placebo and significant enough to establish a clinically relevant effect of the extracts for controlling symptoms associated with cerebral insufficiency. A comparison of the eight best trials of Ginkgo extracts with five trials of ergoloid mesylates (Hydergine®) indicated a similar efficacy of both products as therapy for cerebral insufficiency [23].

In their meta-analysis, Letzel *et al.* [124] considered 44 randomized, double-blind, placebo-controlled studies of Ginkgo biloba extracts (25 trials), nimodipine (9 trials) and tacrine (10 trials), all of which can be designated as "nootropics". Studies with EGb 761 and nimodipine were related to patients with AD-type, multi-infarct or mixed forms of dementia; those with tacrine included only

patients with AD-type dementia. It was concluded that all three substances showed clinical efficacy at the psychopathological, psychometric and behavioral levels.

The meta-analysis conducted by Oken *et al.* [24] concerns the effects of EGb 761 on objective measures of cognitive function in patients with AD-type dementia. More than 50 articles were identified for this analysis, but only four of these met all of the stringent inclusion criteria. These studies [126,137,146,147] involved 212 subjects in each of the EGb 761-treated and placebo-treated groups, and the patients were diagnosed as having "mild-to-moderate" dementia of the AD-type. This analysis led to the conclusion that there is a small, but significant, effect of 3- to 6-month treatment with 120-240 mg/day of EGb 761 on objective measures of cognitive function in AD. An overall modest "effect size" of 0.40 ($p < 0.0001$) was detected which translates into a 3% difference in the Alzheimer's Disease Assessment Scale-cognitive sub-test. This effect size is comparable to the one obtained in the trial on donepezil (Aricept®) conducted by Rogers *et al.* [156]. No significant adverse events occurred in the clinical trials. Oken *et al.* [24] have recommended that a Phase II study should be conducted to find the optimal treatment regimen (dose) for EGb 761, and such a study is already being performed.

The most recent meta-analysis, conducted by Ernst and Pittler [27], was aimed at systematically reviewing the clinical evidence of EGb 761 preparations as a symptomatic treatment for dementia. Computerized literature searches were performed to identify all double-blind,randomized, placebo-controlled trials that assessed clinical end-points of EGb 761 as a treatment for dementia. Nine double-blind, randomized,placebo-controlled trials that met inclusion criteria [126,145-147,157-161] were reviewed. The analysis revealed that EGb 761 is more effective than placebo as a treatment for dementia, an effect that was associated with few, generally mild, adverse side effects. It was concluded that these findings are encouraging and warrant large scale confirmatory and comparative trials.

Collectively, the information gained from these meta-analyses indicates that chronically-administered EGb 761 is useful in treating certain disturbances of cerebral function, including the neurosensory and other symptoms of "mild-to-moderate" dementia of the AD, multi-infarct and mixed types.

CONCLUDING REMARKS

A broad spectrum of pharmacologicalactivities derives from the constituents of EGb 761, each of which would be expected to exert a different effect(s) when it is administered alone as opposed to when it is given together with the other constituents of the extract. Flavonoids (including proanthocyanidins), terpenoids (ginkgolides, bilobalide), organic acids and certain other constituents of EGb 761 likely all play roles in mediating its therapeutic effects. Thus, in a sense, EGb 761 can be viewed as a type of "combination therapy" since it acts as an antioxidant/free radial-scavenger, anti-ischaemic, anti-inflammatory agent with beneficial influences on synaptic remodelling and restoration of age-related deficiencies in central neurotransmitters systems.

Several pharmacological actions of EGb 761 that have been demonstrated in basic studies appear to deserve special attention upon considering its use as therapy for dementias of the AD, multi-infarct and mixed types and other age-associated neurological disorders. These are: its free radical-scavenging and antioxidant effects, its effects on glucose utilization, its improvement of mitochondrial function, its action of restoring age-related decreases in the densities of central neurotransmitter receptors, its "anti-stress" effect, its effect of enhancing recovery of function after traumatic brain lesions, and its effects on learning, memory and neuronal plasticity.

With specific regard to its metabolic, neuroprotective and "anti-stress" actions, certain distinct terpene trilactone constituents of EGb 761 have been shown to act on specific molecular targets. The in vitro results of Chandrasekaran [48] showed a gene-regulatory effect of bilobalide, but no effect of ginkgolide B, in relation to stimulation of the mtDNA-encoded COX III subunit of cytochrome c oxidase. This specific action of bilobalide indicates a molecular target that is implicated in the capacity of EGb 761 to preserve oxidative phosphorylation. The ex vivo results of Amri *et al.* [65] showed that the ginkgolides A and B, but not bilobalide, suppressed the down-regulation of the mitochondrial PBR of the adrenal cortex and its mRNA. From these latter results, it may be ascertained that both the "anti-stress" and neuroprotective effects of EGb 761 which are evident in the CNS may involve a peripheral effect of the extract on glucocorticoid synthesis by the adrenal glands that is mediated by an action of its ginkgolide constituents.

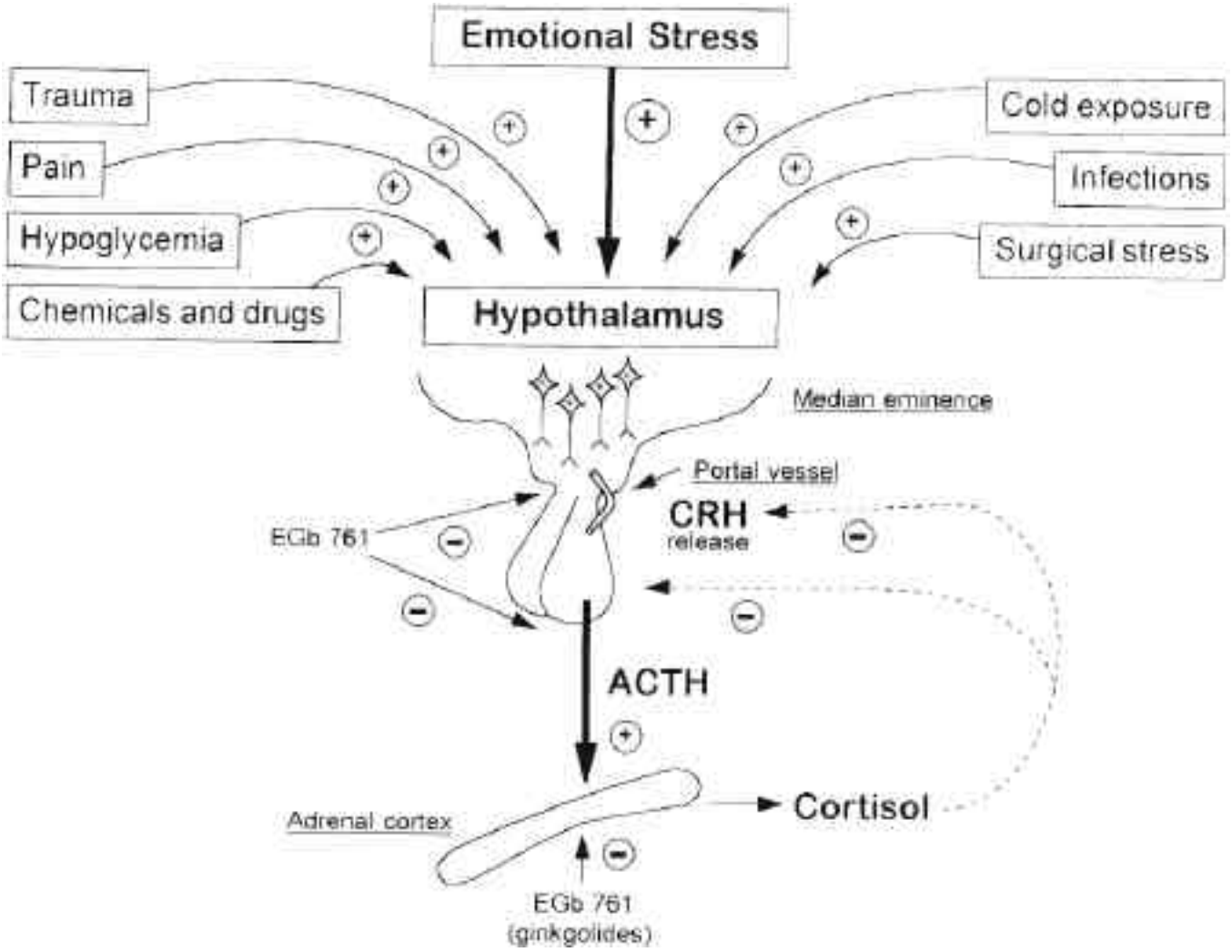

Fig. 6. Hypothetical diagram of the "anti-stress" effect of EGb 761. Emotional stress and various other stressors activate the hypothalamo-pituitary-adrenal axis (stress axis), an effect that initially involves CRH release from the hypothalamus, leading to increased synthesis and release of glucocorticoids at the level of the adrenal cortex. In the normal human being, brain receptors monitor the glucocorticoid concentration of the blood, and their activation by an excess in circulating glucocorticoids can lead to a decrease in blood glucocorticoid concentration (via a negative feedback mechanism) whenever it is increased by stress and could become deleterious. However, patients afflicted with AD or certain other diseases, or even some healthy humans subjected to excessive stress, may have a hyporesponsive stress axis and excessive cortisol in the blood, which could have deleterious consequences. EGb 761, via its ginkgolide constituents, down-regulates (reduces the expression of) the peripheral benzodiazepine receptor (PBR) of the adrenal cortex, thereby suppressing the synthesis (and release) of glucocorticoids (cortisol in the human; corticosterone in the rat; see [65]. However, the total EGb 761 preparation would be required for therapeutic purposes since the increased release of CRH and/or ACTH that would occur in response to this effect of the ginkgolides of decreasing circulating glucocorticoids (via suppression of the negative feedback mechanism), and which could have detrimental consequences, appears to be inhibited by other constituents of the extract (see [116]). (Original unpublished drawing).

With specific regard to AD, the results of Chandrasekaran *et al.* [48] are of interest since COX subunits of Complex IV of the oxidative phosphorylation system have been implicated in the pathogenesis of this disease, which is also characterized by impaired brain oxidative metabolism. The results of Amri *et al.* [65] gain added impetus when viewed in light of the increased activity of the hypothalamo-pituitary-adrenal axis that has been reported to occur in AD patients [162]. Thus, ginkgolide constituents of the extract may be involved in maintaining low levels of glucocorticoids, and another constituent(s) may inhibit pituitary ACTH or hypothalamic CRH release; see Fig. (**6**). These latter results also support recent clinical trials which have indicated that EGb 761 treatment benefits patients afflicted with both AD and depression [163], and in this

regard, it seems noteworthy that both AD and depression have been linked to abnormal function of the hypothalamo-pituitary-adrenal axis [164]. AD is characterized by impaired reactivity of this stress response system, leading to increased and sustained glucocorticoid (cortisol, in humans) release, and eventually to hippocampal damage [99], and it has been postulated that abnormalities in the responsiveness of this system to stress may actually predispose individuals to both AD and depression [115]; see Fig. (**6**).

With further regard to the effects of stress at the human level, Sapolsky [165] has mentioned that sustained stress can have many pathological effects, and that cortisol is among the molecules that mediate such effects, and he recalls that excessive exposure to glucocorticoids adversely affects rodent brain, especially the hippocampus which is vital to learning and memory and which possesses high concentrations of glucocorticoid receptors. He also mentions new evidence which indicates that glucocorticoids may damage human brain; i.e., about half of the depressive patients studied were found to secrete abnormally high amounts of glucocorticoids, a finding that coexisted with bilateral hippocampal atrophy in these patients. Patients with Cushing's syndrome who overproduce glucocorticoids also show bilateral hippocampal atrophy and an impairment of hippocampus-dependent cognition that is correlated with the extent of glucocorticoid hypersecretion. Hippocampal atrophy has also been correlated with post-traumatic stress disorder (PTSD) in Vietnam combat veterans and in adults with PTSD associated with childhood abuse. Although these results at the human level are not entirely conclusive, e.g., with respect to whether or not glucocorticoids actually mediate the hippocampal atrophy, such changes occurred months to years after the trauma and at times when patients did not hypersecrete glucocorticoids, implying that they could represent irreversible neuronal loss. Also, as Sapolsky [165] himself mentions, there is a problem of causality: A small hippocampus could be the cause, rather than a consequence, of the trauma or stressor in these studies, except perhaps for Cushingoid patients or victims of child abuse. Sapolsky [165] also maintains that both therapeutic and experimental administration of glucocorticoids to humans causes memory impairment [see also 99].

The aforementioned clinical studies and meta-analyses have revealed that EGb 761 is beneficial in treating patients exhibiting the various clinical stages of progressive degenerative types of dementia such as AD, as well as multi-infarct and mixed forms of dementia. The significance of such actions is underscored upon considering that regionalized neuronal death, possibly related to age-associated increases in oxidative stress, may underlie the pathology of AD and other dementias. Thus, it seems particularly noteworthy that constituents of EGb 761 can act as free radical-scavengers and antioxidants which theoretically could prevent or delay the onset or progression of the pathology, regardless of the particular risk factors involved. It is also noteworthy that EGb 761 (via its ginkgolide constituents) can down-regulate the PBR and thereby decrease the level of circulating glucocorticoids. Since both oxidative stress and glucocorticoid excess appear to contribute to the decreased glucose transport and utilization, neurodegeneration, membrane damage (which probably occurs via increased lipid peroxidation), excessive amyloid deposition and the associated cognitive decline that occurs in AD patients, it follows that EGb 761 could be useful in delaying the onset and slowing the progression of this disease and other forms of dementia.

A scheme of the various risk factors that might contribute to the development of AD and their relationship to ROS that might damage brain tissue, as well as the influence of the antioxidant effects of EGb 761, is provided in Fig. (**7**). Harman [81] has postulated that mitochondrial dysfunction involving impaired oxidative phosphorylation and excessive production of ROS (e.g., $O_2^{-\bullet}$, H_2O_2) may contribute to the pathogenesis of AD. The free radical-scavengingand anti-lipoperoxidative effects of EGb 761 (which would preserve membrane functional integrity), as well as its effects of improving CBF (which would beneficially influence oxygen consumption and intermediary metabolism), could also be involved in mediating its stress-alleviating action. Its effect of restoring age-related decreases in membrane receptor density, shown in rat neocortex and hippocampus for α_2-adrenoceptors and in rat neocortex for 5-HT_{1A} receptors may also play a significant role in relation to overcoming stress; see above and Fig. (**1**). Regardless of the exact flavonoid and terpene trilactone constituents of EGb 761 that are involved in mediating its free radical-scavenging and antioxidant actions, these actions likely play significant roles in relation to the therapeutic effects of the extract that are observed in the clinical setting. Conditions that are treated with EGb 761-containing products, such as age-associated neurodegenerative diseases (e.g., AD), cerebrovascular insufficiency and cerebral ischaemia, all involve free radicals, related ROS and free radical-induced lipid peroxidation.

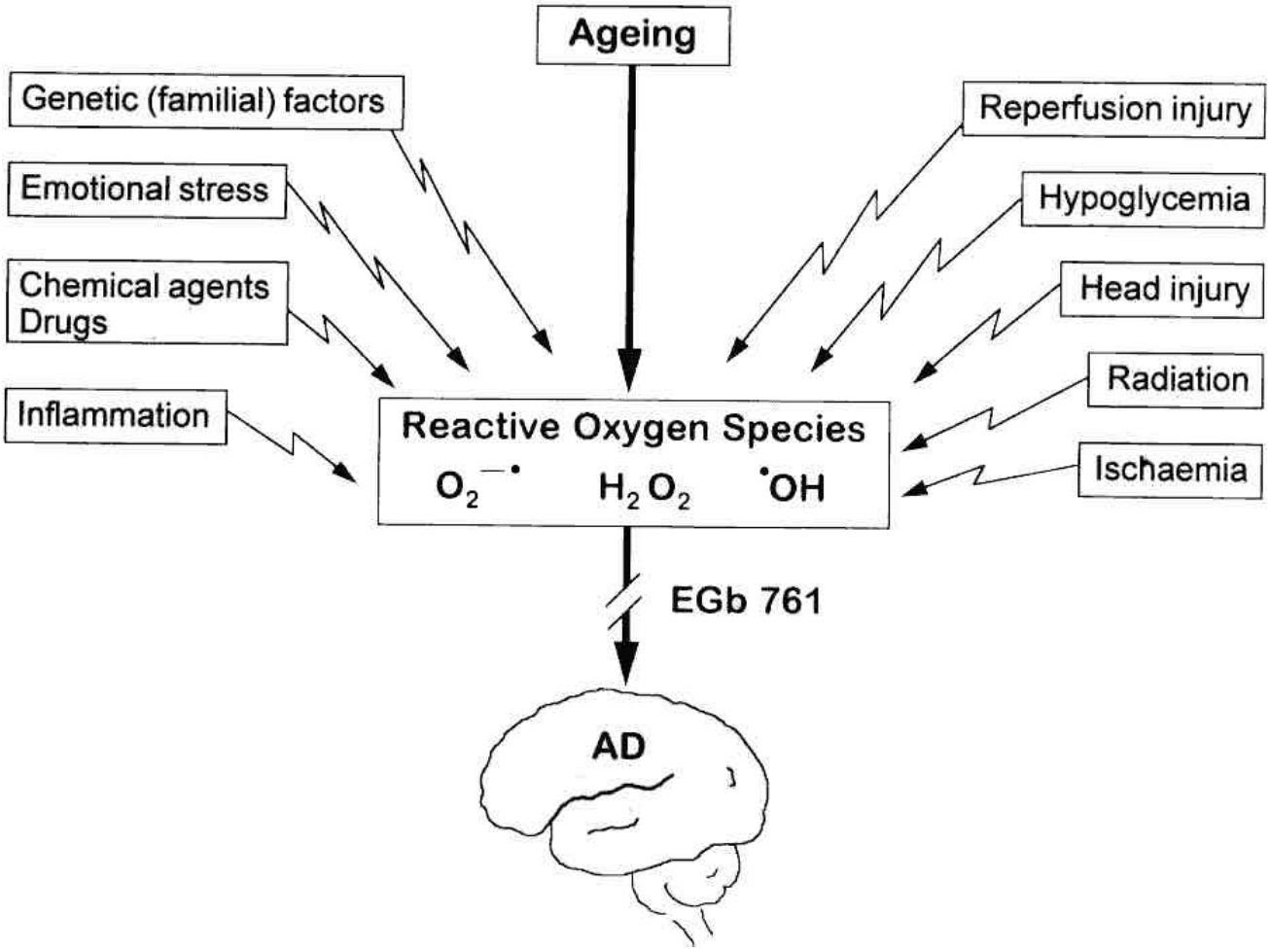

Fig. 7. Hypothetical diagram showing that various risk factors for the development of Alzheimer's disease (AD), ageing being the most important, may all be channeled through a mechanism involving excessive production of potentially detrimental reactive oxygen species (ROS). Treatment with EGb 761, which has free radical-scavenging and antioxidant properties, could delay the progression of this disease. (Original unpublished drawing).

This synopsis of basic and clinical studies of the effects of EGb 761 on CNS mechanisms supports the clinical use of the extract in cases of "mild-to-moderate" dementia of AD, multi-infarct and mixed types, especially in elderly patients [see also 3,21,24,27]. This analysis indicates further that slightly lower doses of EGb 761 may also be useful in treating cognitive impairment and depressive symptoms in ageing patients who are not afflicted with Alzheimer's disease or other severe types of cerebral pathology, therapeutic actions which would be related to the "cognition-enhancing" and "anti-stress" properties of the extract. It is maintained that the total EGb 761 product is required for therapeutic benefit, especially with respect to multifactorial disease states such as AD. The therapeutic use of EGb 761-containing products should increase as the population of aged individuals expands, since employment of methods of preventive medicine to improve the "quality of life" of the elderly should become increasingly more important.

ACRONYMS AND ABBREVIATIONS

AAPH	=	2,2'-Azobis (2 amidinopropane) hydrochloride
ACTH	=	Adrenocorticotrophic hormone
AD	=	Alzheimer's disease
ADAS-Cog	=	Alzheimer's Disease Assessment Scale-Cognitive subscale
ADP	=	Adenosine diphosphate
α_1-Adrenoceptor	=	Adrenergic receptor, subtype α_1

α_2-Adrenoceptor	=	Adrenergic receptor, subtype α_2
AMP	=	Adenosine monophosphate
ApoE	=	Apolipoprotein E
ATP	=	Adenosine triphosphate
ATP-ase	=	Adenosine triphosphatase
BBB	=	Blood-brain barrier
B_{max}	=	Maximal binding capacity, or density of binding sites
CaMKII	=	Ca^{2+}/calmodulin-dependent protein kinase II
CBF	=	Cerebral blood flow
β-CCE	=	Ethyl β-carboline-3-carboxylate
CEEG	=	Computer EEG
CGI	=	Clinical global impressions
CGIC	=	Clinical global impression of change
CNS	=	Central nervous system
COX	=	Cytochrome c oxidase
COX III	=	COX III subunit of cytochrome oxidase
CP 205	=	Extract corresponding to EGb 761 but devoid of terpene trilactone constituents
CRH	=	Corticotrophin-releasing hormone
Cyclic-AMP	=	Adenosine 3', 5'-cyclic phosphate
DA	=	Dopamine
DMSO	=	Dimethyl sulfoxide
DNA	=	Deoxyribonucleic acid
DOPAC	=	3,4-Dihydroxyphenylacetic acid
[^{3}H]8-OH-DPAT	=	[^{3}H]8-hydroxy-2(di-n-propylamino)tetralin
DSM	=	Diagnostic and statistical manual for mental disorders
EDRF	=	Endothelium-derived relaxing factor(s)
EEG	=	Electroencephalogram
EGb 761	=	Extract 761 of the leaves of Ginkgo biloba
GABA	=	γ-Aminobutyric acid
GCES	=	Geriatric clinical evaluation scale
GERRI	=	Geriatric evaluation by relative's rating instrument
H_2O_2	=	Hydrogen peroxide
HACU	=	High-affinity [^{3}H]choline uptake
HPLC	=	High-pressure liquid chromatography
5-HT	=	5-Hydroxytryptamine, or serotonin
iipMF	=	Infrapyramidal mossy fiber
ITT	=	Intent-to-Treat analysis
K_D	=	Apparent dissociation constant, or binding constant
K_i	=	Inhibitor concentration required to decrease ligand binding by 50%
MAO	=	Monoamine oxidase
mRNA	=	Messenger RNA
mtDNA	=	Mitochondrial DNA
NAB	=	Nurembery age observation scale ("Nurnberger-Alters-Beobachtungsskala")
NE	=	Norepinephrine
NGF	=	Nerve growth factor
nNOS	=	Neuronal NO-synthase
NO	=	Nitric oxide
$^{\bullet}N=O$	=	Nitric oxide free radical
$O_2^{-\bullet}$	=	Superoxide anion radical
$OH^{\bullet}$	=	Hydroxyl radical
PBR	=	Peripheral benzodiazepine receptor

PET = Positron emission tomography

P_i = Inorganic phosphate

PK11195 = 1-(2-chlorophenyl)-N-methyl-(1-methyl-propyl)-3-iso-quinolinecarboxamide

QPEEG = Quantitative Pharmaco-EEG method

RNA = Ribonucleic acid

ROS = Reactive oxygen species

RPE cells = Retinal pigmented epithelial cells

S.E.M. = Standard error of the mean

SCAG = Sandoz Clinical assessment-geriatric test

SKT = "Syndrom Kurz Test"

StAR = Steroidogenic acute regulatory protein

TBPT = t-butylbicyclophosphorothionate

TET = Triethyltin

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