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Aging Modulates Nitric Oxide Synthesis and cGMP Levels in Hippocampus and Cerebellum

Effects of Amyloid β Peptide

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ABSTRACT

The biological roles of nitric oxide (NO) and cGMP as inter- and intracellular messengers have been intensively investigated during the last decade. NO and cGMP both mediate physiological effects in the cardiovascular, endocrinological, and immunological systems as well as in central nervous system (CNS). In the CNS, activation of the *N*-methyl-D-aspartic acid (NMDA) type of glutamatergic receptor induces Ca^{2+}-dependent NOS and NO release, which then activates soluble guanylate cyclase for the synthesis of cGMP.

Both compounds appear to be important mediators in long-term potentiation and long-term depression, and thus may play important roles in the mechanisms of learning and memory. Aging and the accumulation of amyloid β (Aβ) peptides are important risk factors for the impairment of memory and development of dementia. In these studies, the mechanism of basal- and NMDA receptor-mediated cGMP formation in different parts of adult and aged brains was evaluated. The relative activity of the NO cascade was determined by assay of NOS and guanylate cyclase activities. In addition, the effect of the neurotoxic fragment 25–35 of Aβ (Aβ) peptide on basal and NMDA receptor-mediated NOS activity was investigated. The studies were carried out using slices of hippocampus, brain cortex, and cerebellum from 3- and 28-mo-old rats.

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Aging coincided with a decrease in the basal level of cGMP as a consequence of a more active degradation of cGMP by a phosphodiesterase in the aged brain as compared to the adult brain. Moreover, a loss of the NMDA receptor-stimulated enhancement of the cGMP level determined in the presence of cGMP-phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) was observed in hippocampus and cerebellum of aged rats. However, this NMDA receptor response was preserved in aged brain cerebral cortex. A significant enhancement of the basal activity of NOS by about 175 and 160% in hippocampus and cerebellum, respectively, of aged brain may be involved in the alteration of the NMDA receptor response. The neurotoxic fragment of Aβ, peptide 25–35, decreased significantly the NMDA receptor-mediated calcium, and calmodulin-dependent NO synthesis that may then be responsible for disturbances of the NO and cGMP signaling pathway.

We concluded that cGMP-dependent signal transduction in hippocampus and cerebellum may become insufficient in senescent brain and may have functional consequences in disturbances of learning and memory processes. Aβ peptide accumulated during brain aging and in Alzheimer disease may be an important factor in decreasing the NO-dependent signal transduction mediated by NMDA receptors.

Index Entries: Nitric oxide; guanylate cyclase; cGMP; NMDA receptors; glutamate receptors; hippocampus; cerebellum; β-amyloid peptide; aging; calcium; calmodulin; phosphodiesterase; signal transduction; cerebral cortex.

Abbreviations: Aβ, β-amyloid peptide; CNS, central nervous system; NMDA, *N*-methyl-D-aspartic acid; APP, β-amyloid precursor proteins; NOS, nitric oxide synthase; NO, nitric oxide.

INTRODUCTION

The activation of excitatory amino acid receptors in the central nervous system (CNS) is transduced by several transmembrane signaling mechanisms, including nitric oxide synthase (NOS) for production of NO and guanylate cyclase for synthesis of intracellular cyclic GMP (Gally et al., 1990; Hu and El-Fakahany, 1993; Vincent, 1995). Stimulation of guanylate cyclase in cerebellar granule cells constituted the first description of a role for NO in the neurons (Garthwaite et al., 1988). Several years ago, L-arginine was identified as an endogenous activator of soluble guanylate cyclase in brain tissue (Deguchi, 1977). NO synthesis occurs as a product of the conversion of L-arginine into citrulline by NOS (Knowles et al., 1989).

At least four sources of NO influence the CNS. A constitutive NOS, which is regulated by a calcium calmodulin-dependent mechanism, is

responsible for NO originating from the endothelium, from cerebral perivascular nerve fibers, and from neurons and glia. NO is also produced by an inducible, noncalcium-dependent type of NOS in macrophages, microglia, and astrocytes in a variety of pathological states (Forstermann et al., 1991). NO has became established as a retrograde messenger. The occurrence of the NO signaling pathway in vascular endothelium and other cells indicates that this is a widespread transduction mechanism whose major function in each case is the stimulation of soluble guanylate cyclase (Miki et al., 1977; Deguchi and Yoshioka, 1982; Knowles et al., 1989).

The role of cGMP in the CNS is still poorly defined except for its crucial involvement in retinal phototransduction. By analogy to vascular smooth muscle cells, the cGMP thus generated antagonized the rise in $[Ca^{2+}]_i$ as part of a negative feedback or desensitization response. This hypothesis is supported by reports that cGMP is excitoprotective (Garthwaite and Garthwaite, 1988). cGMP can reduce calcium currents in hippocampus slices (Doerner and Alger, 1988). cardiac myocytes (Wahler et al., 1990; Mery et al., 1991). and chick ciliary ganglion neurons (Meriney et al., 1994). cGMP can decrease the activity of phospholipase C in vascular preparations and smooth muscle cell cultures as determined by the decreased formation of inositol phosphates. This effect appears to be mediated by a cGMP-dependent protein kinase. These biochemical events mediated by cGMP may lower the concentration of cytosolic free calcium (Hirata et al., 1990; Wang and Robinson, 1997).

cGMP is also produced by the particulate guanylate cyclase associated with receptors for the various natriuretic peptides that are differentially expressed in neurons and glia (Brown and Czarnecki, 1990; Hosli and Hosli, 1992). The cGMP content is also increased by β-amyloid precursor proteins (APP), and cGMP is capable of mimicking the ability of soluble APP to lower $[Ca^{2+}]_i$ in hippocampal neurons (Barger et al., 1995). In addition, the apparent modulation of NMDA receptors by APP/cGMP may have important implications for *N*-methyl-D-aspartic acid (NMDA)-dependent phenomena, including the refinement of synaptic connections during development (Constantine-Paton et al., 1990). and synaptic plasticity (Malenka, 1994). More direct evidence for the involvement of cGMP in long-term potentiation and depression was reported recently by Zhuo et al. (1994). Quite recently, Bernabeu et al. (1997). demonstrated involvement of a hippocampal cGMP-dependent protein kinase cascade in memory consolidation. Aging seems to decrease the basal and stimulated release of endothelium-derived NO in blood vessels (Luscher et al., 1992). However, little is known about the effect of aging on NO and cGMP signaling in the CNS.

In the current work, the studies were focused on the modulatory mechanism of aging on basal- and NMDA receptor-evoked synthesis of NO and cGMP in different parts of the brain. In addition, the effect of

Aβ peptides on the basal- and NMDA receptor-mediated NOS was investigated. A preliminary report on this work has appeared (Chalimoniuk and Strosznajder, 1997).

MATERIALS AND METHODS

Materials

The Animal Farm, Lomna, Poland, supplied male Wistar rats, adult (3 mo, 200–240 g) and aged (28 mo, 300–350 g). L-[^{3}H]arginine, cyclic [^{3}H]GMP, and enzyme immunoassay kits were purchased from Amersham, Buckinghamshire, England. 7-Nitroindazol (7-NI) and dizocilpine maleate (MK-801) were obtained from Research Biochemicals International, Natick, MA. NMDA, DL-2-amino-5-phosphonopentanoic acid (APV), *N*-nitro-L-arginine (NNLA), 3-isobutyl-1-methylxanthine (IBMX), and all other reagents were obtained from Sigma, St. Louis, MO. Dowex AG50W-X8 (Na^+ counterion) cation-exchange resin was purchased from Serva, Heidelberg, Germany.

Preparation of Slices from Different Parts of the Brain

Male Wistar rats were used for the experiments. The rats were decapitated, and the brain was removed rapidly. The hippocampus, cerebellum, and brain cortex were isolated, and then cut at 0.35-mm intervals in both the sagittal and coronal planes using a McIlwain tissue chopper. The slices were dispersed in 15 mL Krebs-Henseleit buffer containing: 124 m*M* NaCl, 5 m*M* KCl, 26 m*M* $NaHCO_3$, 1.24 m*M* NaH_2PO_4, 2.4 m*M* $CaCl_2$, and 10 m*M* glucose, and centrifuged at 1100*g* for 15 min. The pellets were dispersed in Krebs-Henseleit buffer continuously gassed with 95% $O_2$5% CO_2. The slices were preincubated for 90 min at 37°C for metabolic equilibrium as demonstrated in Fig. 1. Then the slices were incubated with NMDA receptor agonist or antagonist or inhibitors of NOS in the absence and the presence of 4 m*M* IBMX, an inhibitor of phosphodiesterase, for 10 min at 37°C before determination of cGMP levels.

Determination of cGMP Level in Brain Slices

The hippocampus, cerebellum, and brain cortex slices were incubated in the absence or presence of the glutaminergic receptor agonist NMDA at 100-μ*M* concentration. In some experiments, 100 μ*M* NNLA or 7-NI, NOS inhibitors, or MK-801 or APV at 10 μ*M*, NMDA receptor antagonists, were added to the medium. The incubation was carried out for 10 min at 37°C in a total volume of 200 μL. The slices were inactivated with 0.2 mL of cold 20% trichloroacetic acid (TCA) by homogenization. The homogenate was centrifuged at 3000 rpm for 5 min at 4°C.

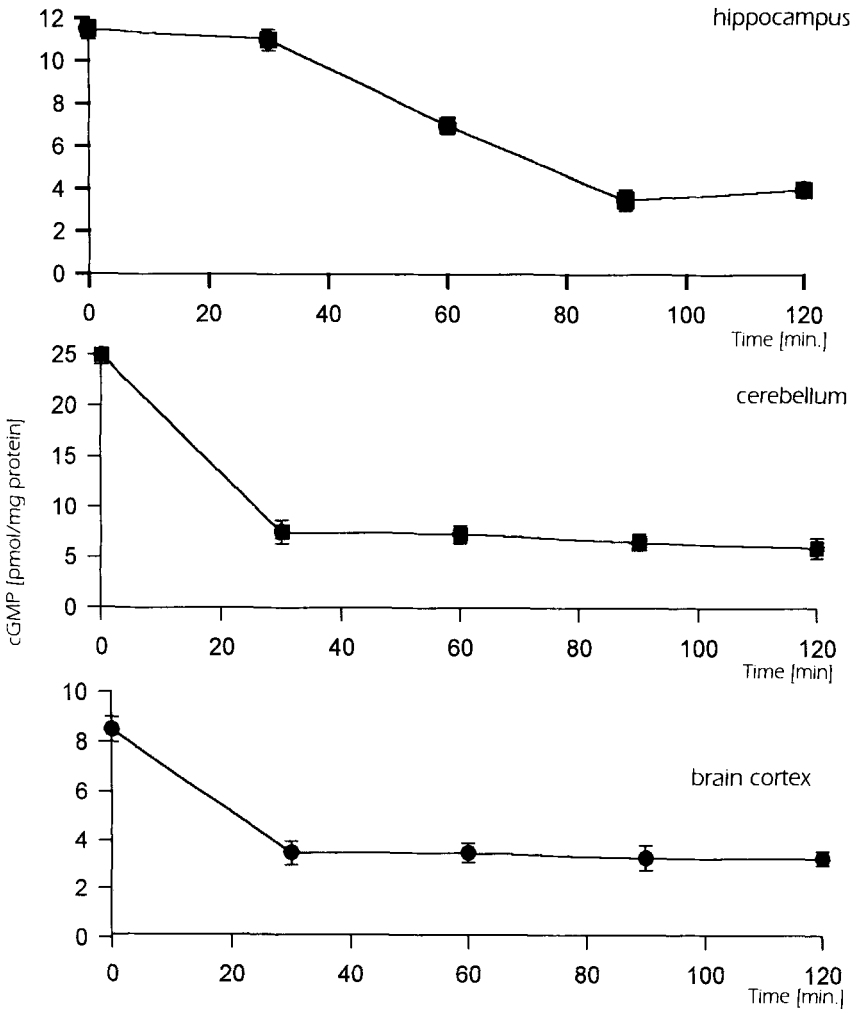

Fig. 1. Effect of preincubation time on cGMP concentration in different parts of adult rat brain. The brain slices were preincubated for 90 min at 37°C in Krebs-Henseleit buffer, pH 7.4, and then were incubated for 10 min at 37°C. The reaction was terminated with 0.2 mL of 20% TCA. Data represent the mean from four experiments with triplicate estimation.

The supernatant was washed four times with water-saturated diethyl ether and neutralized with 1 *M* NaOH to pH 7.4 before determination of the cGMP content using a radioimmunoassay method.

Determination of NOS Activity

NOS activity was determined by using a modified assay system originally described by Bredt and Snyder (1990). Adult and aged rats were decapitated, and the brains were dissected. The desired portions were homogenized in ice-cold 50 m*M* Tris-HCl buffer, pH 7.4, containing

1.15% KCl (w/v), 1 m*M* enthylenediamine-tetra-acetic acid EDTA, 5 m*M* glucose, 0.1 m*M* D,L-dithiothreitol (DTT), 2 mg/L of pepstatin A, 10 mg/L trypsin inhibitor, and 44 mg/L phenylmethylsulfonyl fluoride (PMSF). The homogenate, 200 μg of protein, was incubated for 30 min at 37°C with 10 μ*M* L-[^{3}H]arginine (1 μCi), 1 m*M* reduced nicotinamide adenine dinucleotide phosphate NADPH, 1 μ*M* calmodulin in 50 m*M* HEPES buffer, pH 7.4, containing 1 m*M* DTT, 1 m*M* EDTA, and 2 m*M* $CaCl_2$ in a final volume of 300 μL. The reaction was terminated by addition of 1 mL of ice-cold 100 m*M* HEPES buffer, pH 5.5, containing 10 m*M* EGTA and 500 mg of Dowex AG 50W-X8 (Na^+ counterion) cation exchange resin (Serva), left for 5 min at 0–4°C, and finally centrifuged at 1000*g* for 5 min at 4°C. Aliquots of supernatant fractions were taken for determination of radioactivity in L-[^{3}H]citrulline in a liquid scintillation counter.

The characterization of the NMDA receptor response for NO and cGMP synthesis is presented in Fig. 2.

Determination of Guanylate Cyclase Activity

Guanylate cyclase activity was assayed as described by Bonkale et al. (1995). The hippocampus and cerebellum were homogenized in ice-cold 10 m*M* Tris-HCl buffer, pH 7.6, with a glass-Teflon homogenizer. The reaction medium contained 4 m*M* IBMX and various concentrations of $MgCl_2$-GTP (0.25–4 m*M*). The reaction was performed in a final volume of 300 μL, and was initiated by addition of 200 μg of protein and incubated for 10 min at 37°C, then it was terminated with 200 μL of 20% TCA. Samples were mixed vigorously, and then were centrifuged at 3500 rpm for 15 min at 4°C. The supernatant was washed four times with water-saturated diethyl ether and then neutralized with 1 *M* NaOH to pH 7.4 before determination of cGMP. Fifty-microliter aliquots of the supernatant were assayed for cGMP content using a commercial cGMP enzyme immunoassay kit (Amersham TNP 226). Values for the maximal enzyme activity (V_{max}) and affinity (K_m) were determined from Hanes plot as (S) vs [S/V] of data that were fitted by linear regression.

Determination of the Effect of Amyloid β Peptide 25–35 on Basal and NMDA Receptor-Mediated NOS

The brain slices were preincubated with or without 25 μ*M* Aβ peptide 25–35 for 5 min at 37°C. The slices were then incubated in the absence or presence of 100 μ*M* NMDA for 10 min at 37°C. The activity of NOS was determined in the presence of 2 m*M* $CaCl_2$, 1 μ*M* calmodulin, and other cofactors as described above.

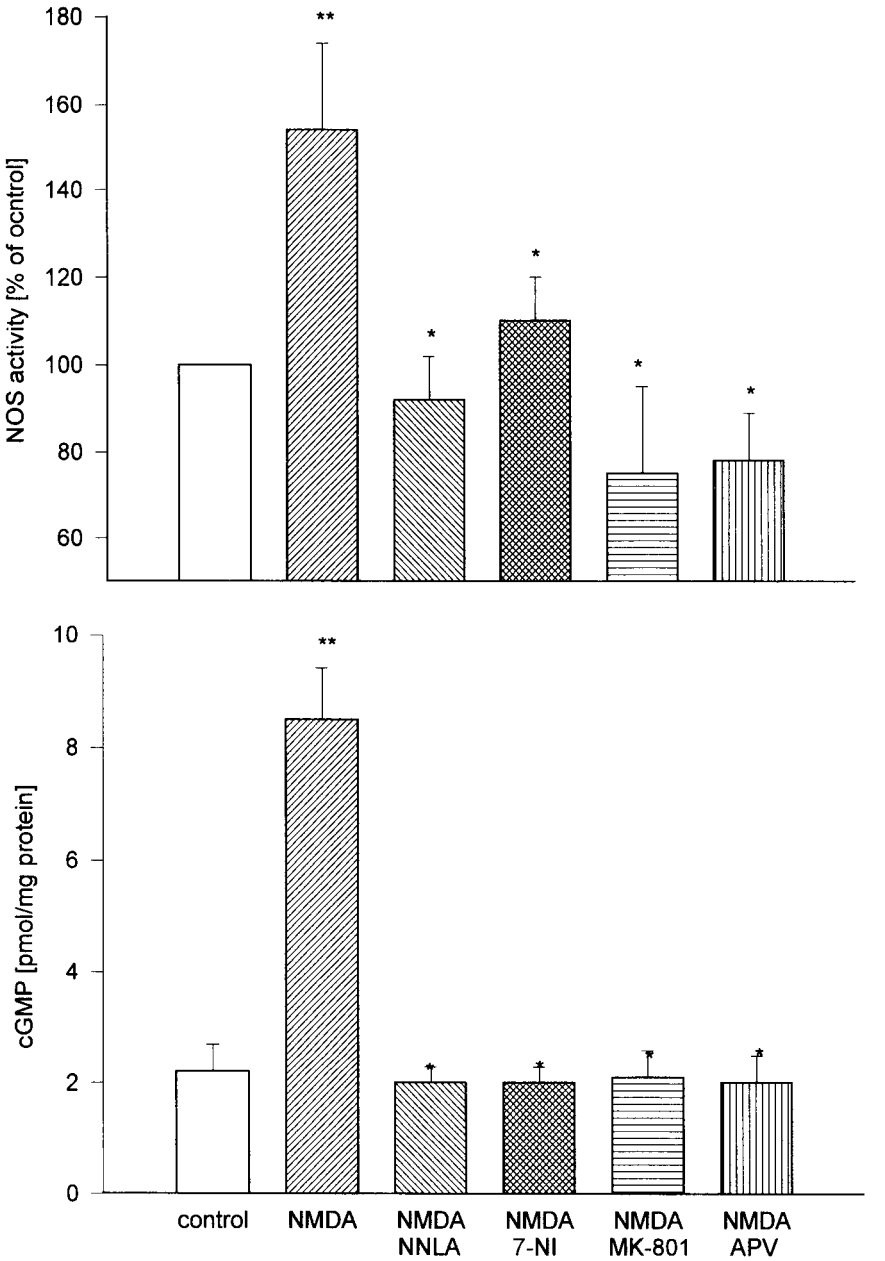

Fig. 2. NMDA receptor-dependent NOS and cGMP synthesis in adult brain cortex slices. The brain slices were preincubated for 90 min at 37°C in Krebs-Henseleit buffer, pH 7.4, then were incubated for 10 min at 37°C in the absence or presence of 100 μ*M* NMDA. Reagent concentrations were 100 μ*M* NNLA, 7-NI, or MK-801, or APV at 10 μ*M*. Data represent the mean ± SD from 4 experiments performed in triplicate. Data were evaluated by one-way ANOVA. Statistical significance was determined by a post-hoc Tukey HSD test. $^{**}p < 0.05$ compared with control, $^{*}p < 0.05$ compared with NMDA.

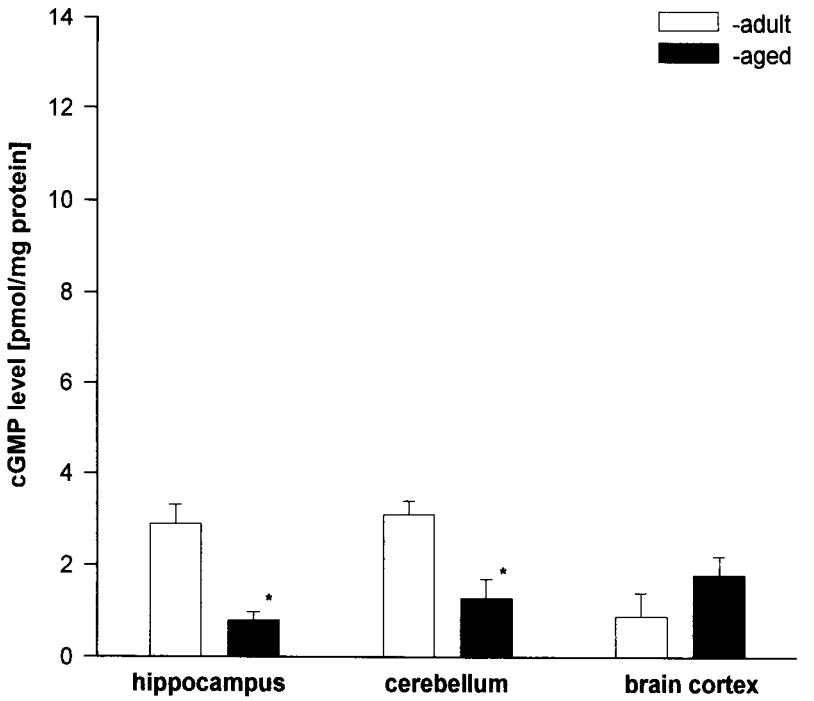

Fig. 3. Basal levels of cGMP in adult and aged hippocampus, cerebellum, and brain cortex slices. The brain slices were preincubated for 90 min at 37°C in Krebs-Henseleit buffer, pH 7.4, and then were incubated for 10 min at 37°C. The reaction was terminated with 0.2 mL of 20% TCA. Data represent the mean ± SD from 4 experiments with triplicate estimation. Data were evaluated by one-way ANOVA. Statistical significance was determined by a post-hoc Tukey HSD test. $*p < 0.05$ compared with adult.

Statistical Analyses

The results are expressed as means ± SD. Data was evaluated by one-way ANOVA, and statistical significance was determined with a post-hoc Tukey HSD test. A p value < 0.05 was regarded as significant.

RESULTS

The cGMP levels were determined in hippocampus, brain cortex, and cerebellum of adult and aged rats. The basal level of cGMP observed in hippocampus and cerebellum of aged rats was significantly lower when compared to adult rats. In aged brain cortex, the cGMP level was similar to that observed in adult brain (Fig. 3). However, when determinations of cGMP were carried out in the presence of the cGMP-phosphodiesterase inhibitor IBMX, a significantly higher level of cGMP was observed in aged brain as compared to adult. These results indicated a higher activity of phosphodiesterase in all investigated parts of aged rat brain (Fig. 4).

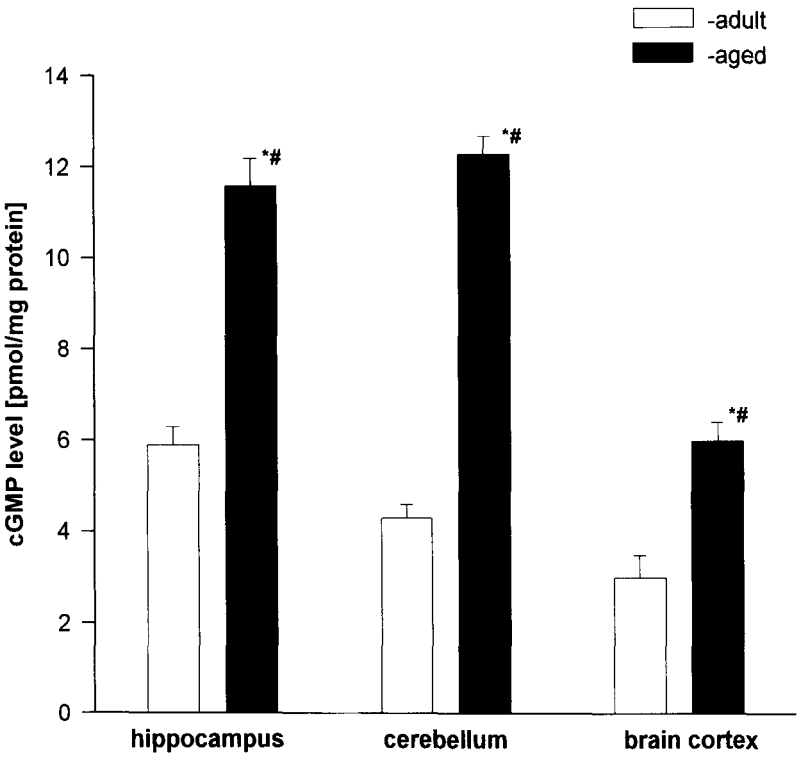

Fig. 4. Effect of phosphodiesterase inhibitor on the level of cGMP in different parts of adult and aged brain. The brain slices were preincubated and then incubated for 10 min at 37°C as described in Materials and Methods in the absence or presence of 4 m*M* IBMX. The data represent mean ± SD of 3 independent experiments with triplicate estimation. Data were evaluated by one-way ANOVA. Statistical significance was determined by a post-hoc Tukey HSD test. $^{\#}p < 0.05$ compared to the basal value, $^{*}p < 0.01$ compared with adult.

On the other hand, a higher activity of guanylyl cyclase was found in aged hippocampus and cerebellum. The enzyme's affinity for substrate was significantly greater in both of these parts of aged brain when compared to the adult brain. The K_m value changed from 47 μ*M* in adult to 23 μ*M* in aged hippocampus and from 78 μ*M* in adult to 32.5 μ*M* in aged cerebellum (Table 1). These data suggested the higher turnover of cGMP in aged brain, however, with more active degradation then biosynthesis.

In further studies, the basal activity of NOS in different parts of adult and aged brain was investigated. Significantly higher NOS activity was observed in aged brain. The activity of NOS was enhanced by about 175, 160 and 125%, respectively, in hippocampus, cerebellum, and brain cortex of aged compared to adult rats (Fig. 5).

For understanding better the mechanism of NO and cGMP synthesis in brain, the release of these messengers was determined during NMDA receptor stimulation. Activation of the glutamatergic NMDA type of receptor in all investigated parts of adult brain enhanced the Ca^{2+}-calmodulin-

Table 1
Guanylate Cyclase Activity in Hippocampus and Cerebellum of Adult and Aged Rats[a]

	V_{max}, (pmol/mg protein)	K_m, μM Mg^{2+}-GTP
Hippocampus		
Adult	12.08 ± 3.4	47 ± 17.6
Aged	15.84 ± 3.7	23 ± 12.4[b]
Cerebellum		
Adult	10.98 ± 4.1	78.1 ± 25.8
Aged	13.65 ± 1.7	32.5 ± 8.2[b]

[a]Data represent the means ± SD from 4 experiments made in triplicate determined from the Hanes plots. Statistical significance was evaluated by one-way ANOVA. A post-hoc Tukey HSD test was used for the comparison of values.

[b]$p < 0.05$ vs the value of adult.

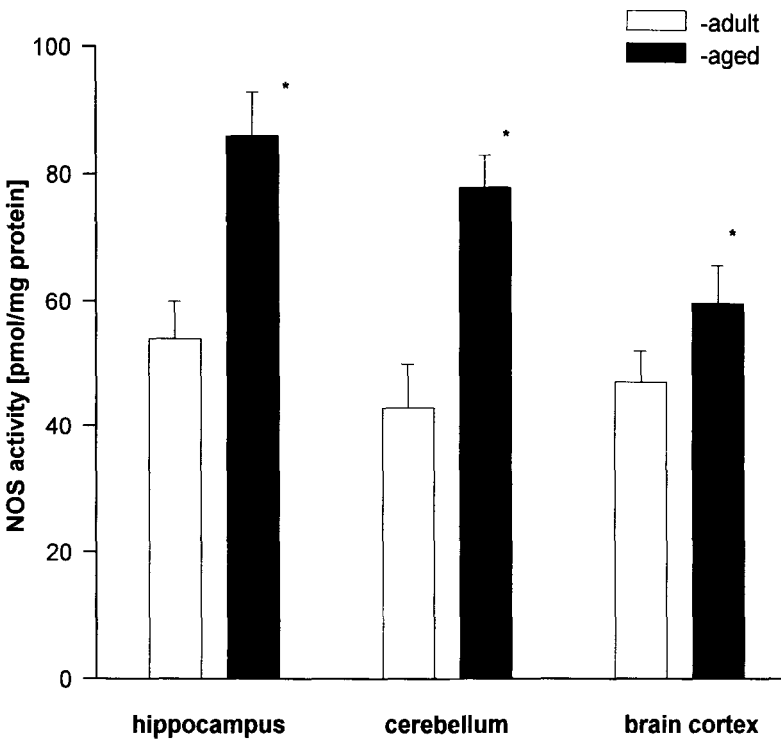

Fig. 5. The basal Ca^{2+}-dependent NOS activities in different parts of adult and aged brain. The enzymic activity was determined in the presence of 2 m*M* Ca^{2+}, 1 μ*M* calmodulin, and other cofactors as described in Materials and Methods. The values represent the mean ± SD from 3–4 experiments with triplicate estimation. Data were evaluated by one-way ANOVA. Statistical significance was determined by a post-hoc Tukey HSD test. *$p < 0.05$ compared with adult.

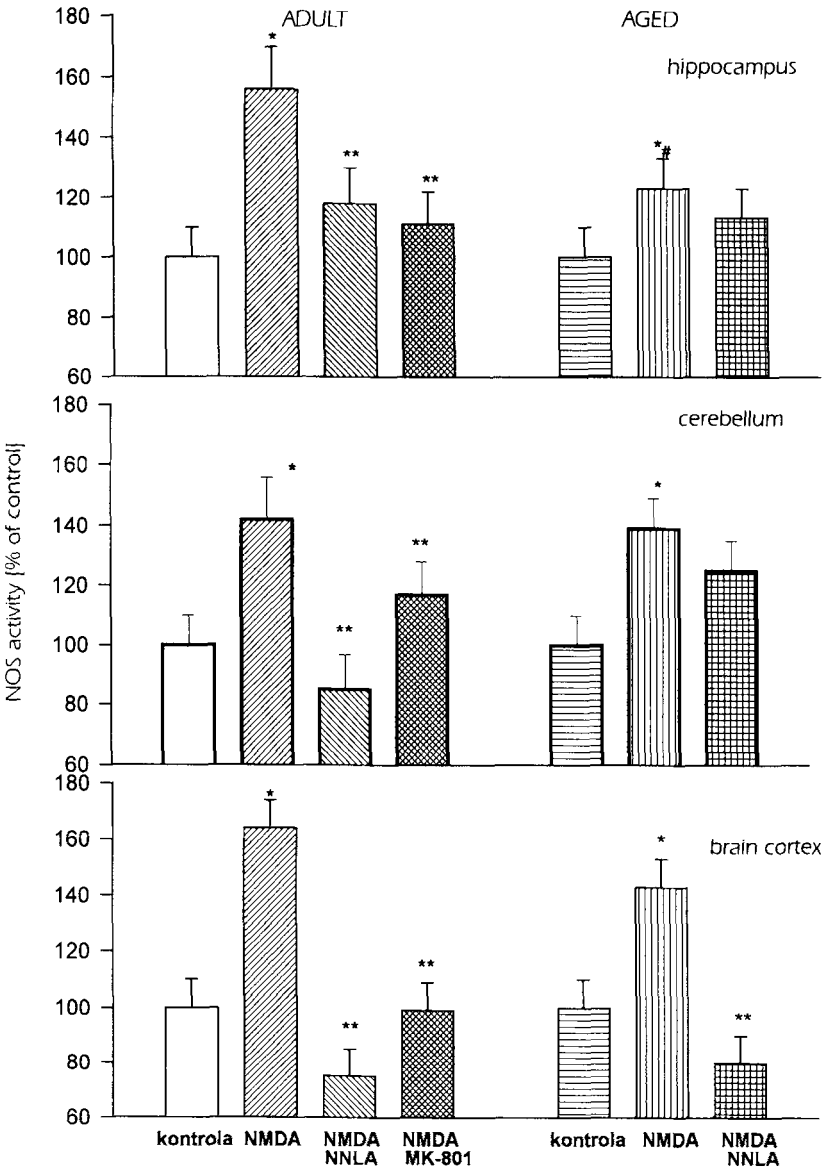

Fig. 6. Effect of NMDA receptor stimulation on NOS activity in different parts of adult and aged brain. NNLA at 100 μ*M* or MK-801 at 10 μ*M* was added into the incubation medium. The brain slices were incubated for 10 min at 37°C in the presence of 100 μ*M* NMDA. The value are means ± SD from 3 experiments carried out in triplicate. Data were evaluated by one-way ANOVA. Statistical significance was determined by a post-hoc Tukey HSD test. $^{*}p < 0.01$ compared with the control value, $^{**}p < 0.01$ compared with NMDA, $^{\#}p < 0.05$ compared with NMDA in adult.

dependent NOS activity. A significantly lower response of the NMDA receptors for NO synthesis was found in senescent brain (Fig. 6). NMDA receptor activation in the adult brain increased the cGMP level by about

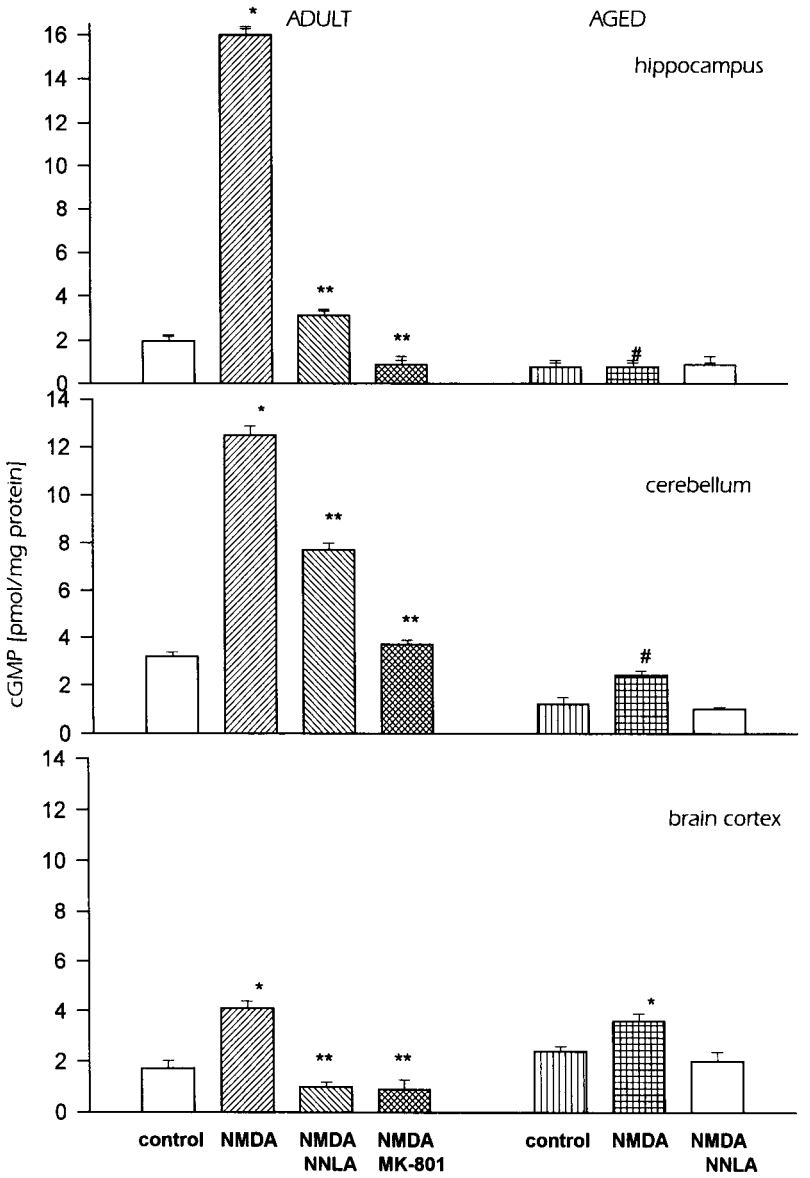

Fig. 7. NMDA receptor stimulation does not lead to the enhancement of cGMP level in hippocampus and cerebellum of aged brain. The brain slices were incubated for 10 min at 37°C in the presence of 100 μ*M* NMDA and in the absence or presence of inhibitors or antagonist. Experimental details were as described in Materials and Methods. The values are means ± SD from 3 experiments with triplicate estimation. Data were evaluated by one-way ANOVA. Statistical significance was determined by a post-hoc Tukey HSD test $^{*}p < 0.05$ compared with control, $^{**}p < 0.01$ compared with NMDA, $^{\#}p < 0.05$ compared with the value of NMDA in adult.

six- to eightfold in hippocampus and cerebellum, respectively, and by about onefold in cerebral cortex when compared with unstimulated conditions (Fig. 7).

The inhibitor of the NMDA receptor-operated Ca^{2+} channel, MK 801, eliminates this receptor-evoked cGMP enhancement in all parts of the brain. The specific inhibitor of both Ca^{2+}-dependent isoforms of NOS, NNLA, suppresses NMDA receptor-mediated cGMP elevation in hippocampus and brain cortex, and decreases by about 50% the level of cGMP in cerebellum (Fig. 7). The inhibitor of phospholipase A_2, quinacrine, also decreased NMDA receptor-dependent elevation of the cGMP level in cerebellum.

In aged hippocampus and cerebellum, the activation of NMDA receptor investigated in the absence (Fig. 7) or presence of phosphodiesterase inhibitor (IBMX) (Fig. 8) does not lead to enhancement of the cGMP level. The NMDA receptor response connected with cGMP synthesis is preserved in aged brain cortex (Figs. 7 and 8). Aβ peptide 25–35 has no significant effect on NOS activity under basal unstimulated condition (Fig. 9). However Aβ 25–35 significantly decreases NMDA receptor-mediated, Ca^{2+}/calmodulin-dependent NOS activity in hippocampus and also in brain cortex (Fig. 9).

DISCUSSION

As compared with the brains of adult rats, we found significantly lower cGMP concentrations in the hippocampus and cerebellum of aged animals. Moreover, NMDA receptor-mediated NO and cGMP synthesis was lower in aged brain. The more pronounced degradation of cGMP by phosphodiesterase in aged brain may be responsible for the lower basal level in hippocampus and cerebellum compared to the adult values. The higher basal activity of Ca^{2+}/calmodulin-dependent NOS and higher NO production in aged brain compared to adult may be involved in the alteration of receptor(s) and/or enzyme(s), including the alteration of NMDA receptor-mediated signal transduction in aged brain. Aging significantly reduced NMDA receptor-dependent NO synthesis. This may subsequently decrease the cGMP signaling pathway in aged brain.

Aging and the accumulation of Aβ peptides are probably the most important risk factors for learning and memory dysfunction, dementia, and Alzheimer disease. Aβ may have a significant effect on NMDA receptor-mediated NO synthesis. The accumulation of Aβ peptides in aged brain and in Alzheimer disease may be an important factor involved in the alteration of NMDA receptor-mediated signaling. Bonkale et al. (1995). have observed reduced NO responsive soluble

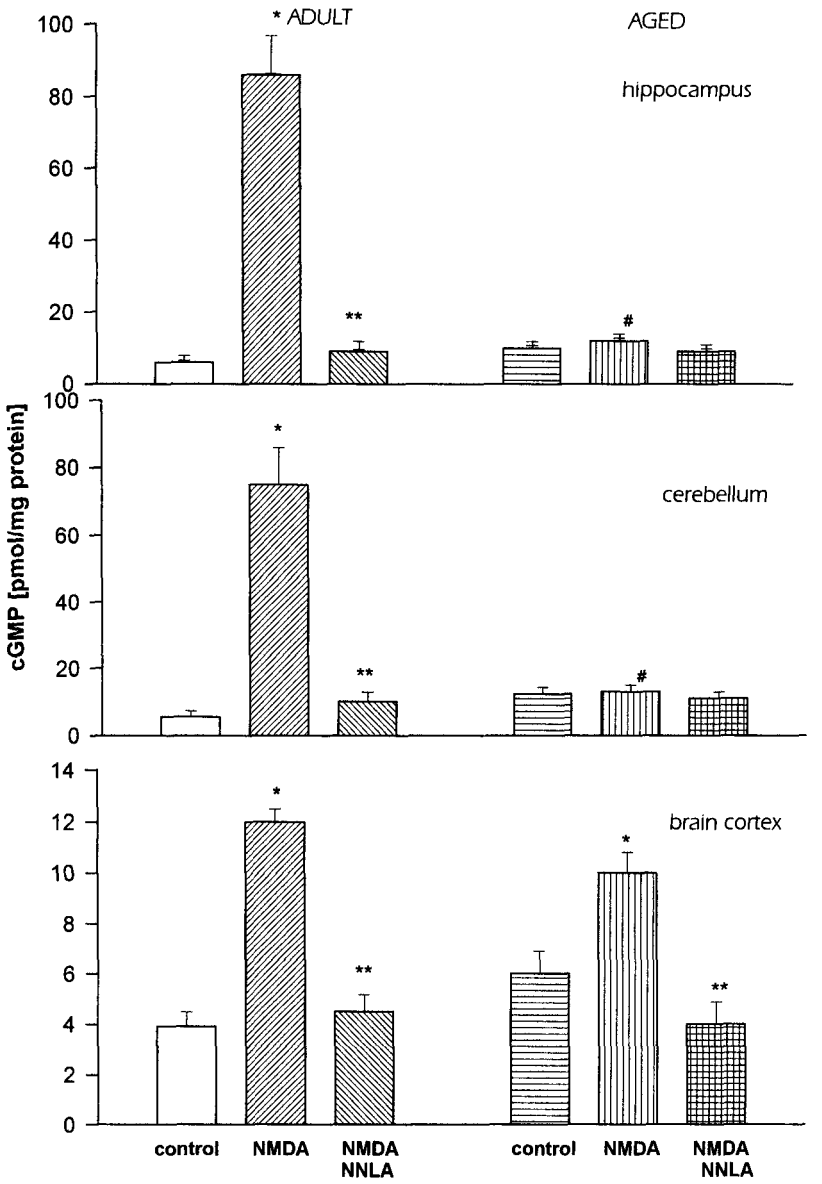

Fig. 8. Effect of NMDA receptor activation on the level of cGMP in the presence of IBMX in different parts of adult and aged brain. The brain slices were incubated for 10 min at 37°C in the presence of 100 μ*M* NMDA and 4 m*M* IBMX. Experimental details were as described in Materials and Methods. The values are means from three experiments with triplicate estimation. Data were evaluated by one-way ANOVA. Statistical significance was determined by a post-hoc Tukey HSD test. $^{*}p < 0.05$ compared with control, $^{**}p < 0.05$ compared with NMDA, $^{\#}p < 0.05$ compared with the value of NMDA in adult.

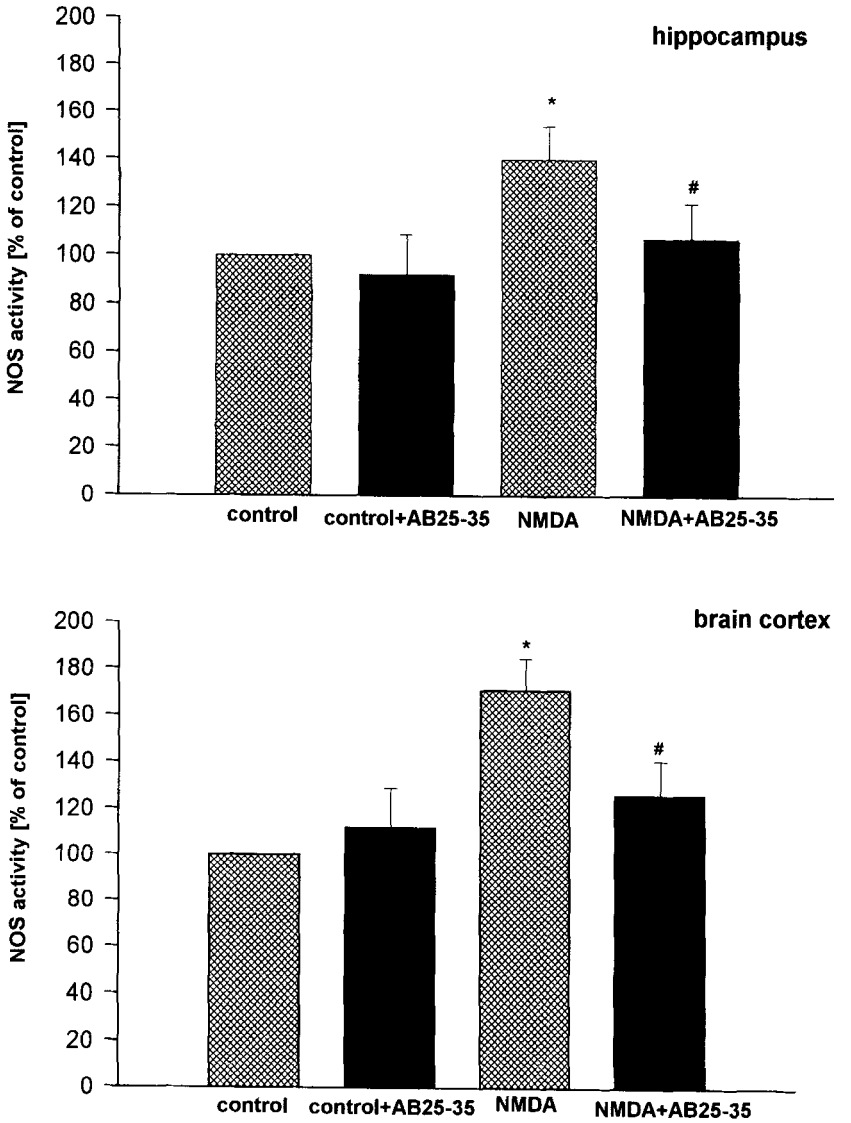

Fig. 9. Aβ 25–35 decreases the NMDA-evoked stimulation of NOS activity in brain cortex and hippocampus. The brain slices were preincubated for 5 min at 37°C with Aβ peptide 25–35 at 25 μ*M* and then were incubated for 10 min at 37°C without and with NMDA at 100 μ*M*. The enzymic activity was determined in the presence of 2 m*M* $CaCl_2$, 1 μ*M* calmodulin, and other cofactors as described in Materials and Methods. The values represent the means ± SD from 5 experiments performed in triplicate. Data were evaluated by one-way ANOVA. Statistical significance was determined by a post-hoc Tukey HSD test. $^{*}p < 0.05$ compared with control, $^{\#}p < 0.05$ compared with NMDA.

guanylyl cyclase activity in the superior temporal cortex of patients with Alzheimer disease.

Excitatory amino acids are thought to play a critical role in such important phenomena as memory and neurotoxicity. The receptor sensitive to NMDA plays a key role in the models of elementary learning, long-term potentiation, and depression (Monaghan et al., 1989; Collingridge and Singer, 1990). Age-related changes of NMDA receptor density have been reported for many species, including humans, and are mainly found in cortical areas and the hippocampus (Tamaru et al., 1991). Stimulation of the NMDA receptor induces NO synthesis, which then activates soluble guanylate cyclase (Garthwaite, 1991). and leads to formation of cGMP. NO has been implicated in neuron–glia interactions, synaptic plasticity, and long-term potentiation and depression (Gally et al., 1990; Garthwaite, 1991; Shibuki and Okada, 1991; Zhuo et al., 1994; Vincent, 1995).

A decrease in NMDA receptor density might be one of the causative factors of cognitive decline with aging. The evidence is less clear regarding to what extent this decline of receptor density is accompanied by similar changes in receptor function. Long-term potentiation involves cGMP (Zhuo et al., 1994). and decreases during aging. The alteration of NO and cGMP synthesis in aged hippocampus and cerebellum that we observed may have important functional implications in the processes of learning and memory.

A primary action of elevated cGMP is the stimulation of cGMP-dependent protein kinase (PKG). PKG phosphorylates substrate proteins to exert its actions (Buisson et al., 1993; Garthwaite and Boulton, 1995; Wang and Robinson, 1997). Remarkably few PKG substrates in brain are known. Examples are DARP-32 protein, phosphatase regulator, dopamine- and cAMP-regulated phosphoprotein, and phospholipase C (Wang and Robinson, 1997). There is growing evidence for a neuronal role of NO and cGMP in the regulation of gene expression in neurons (Johnston and Morris, 1994). APP $[Ca^{2+}]_i$-lowering and excitoprotective effects on target neurons through increases in cGMP level with activation of PKG (Barger et al., 1995; Furukawa et al., 1996). Our results indicate that aging and Aβ peptide significantly decrease NMDA receptor-mediated NO synthesis, which may then be responsible for the decrease in NO-dependent cGMP synthesis.

Activation of a specific receptor may modulate neurotransmitter or hormone secretion through changes in the concentration of cGMP in response to calcium-dependent processes (Antoni and Dayanithi, 1990; White et al., 1993). The endogenous production of NO and cGMP may also be involved in modulation of NMDA receptor function (Manzoni et al., 1992; Barger et al., 1995).

The cerebellum has traditionally been viewed as a structure that contributes primarily to motor control and coordination. However, beginning

in the middle 1980s, anatomical, behavioral, and neurophysiological evidence began to suggest that the role of the cerebellum extends beyond a purely motor domain. There is now a growing consensus that the cerebellum contributes to some aspects of cognition (Fiez, 1996), but the exact process is not known. Circuits connecting the cerebellum and nonmotor cortical areas do exist (Schamahmana, 1994). Also simultaneous repetitive activation of parallel fibers and axons of cerebellar granule cells and climbing fiber axons leads to long-term depression (LTD). NO and cGMP play important roles in the mechanism of LTD (Vincent, 1995).

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