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Cyclic GMP and Nitric Oxide Synthase in Aging and Alzheimer's Disease

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Abstract Cyclic guanosine monophosphate (cGMP) is an important secondary messenger synthesized by the guanylyl cyclases which are found in the soluble (sGC) and particular isoforms. In the central nervous system, the nitric oxide (NO)-sensitive sGC isoform is the major enzyme responsible for cGMP synthesis. Phosphodiesterases (PDEs) are enzymes for hydrolysis of cGMP in the brain, and they are mainly isoforms 2, 5, and 9. The NO/cGMP signaling pathway has been shown to play an important role in the process underlying learning and memory. Aging is associated with an increase in PDE expression and activity and a decrease in cGMP concentration. In addition, aging is also associated with an enhancement of neuronal NO synthase, a lowering of endothelial, and no alteration in inducible activity. The observed changes in NMDA receptor density along with the Ca^{2+}/NO/cGMP pathway underscore the lower synaptic plasticity and cognitive performance during aging. This notion is in agreement with last data indicating that inhibitors of PDE2 and PDE9 improve learning and memory in older rats. In this review, we focus on recent studies supporting the role of Ca^{2+}/NO/cGMP pathway in aging and Alzheimer's disease.

Keyword Aging · Alzheimer's disease · Brain · Cyclic GMP · Memory · Nitric oxide · Phosphodiesterases

Abbreviations

AD	Alzheimer's disease
CNS	Central nervous system
cGMP	Cyclic GMP
PDEs	Phosphodiesterases
PKG	cGMP-Dependent protein kinase G
sGC	Guanylyl cyclase (soluble isoform)
NOS	NO synthase

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Introduction

cGMP is a widely distributed secondary messenger in the mature brain. The level of cGMP is much higher in the brain compared with the peripheral organs and is 20 times higher in the newborn brain compared with a mature one. During aging, cGMP levels in the brain significantly decrease. The final concentration of cGMP in cells depends on the balance of its synthesis via guanylyl cyclase(s) [EC 4.6.1.2] and degradation by PDEs [EC 3.1.4]. cGMP synthesis in the brain occurs mainly by activation of NO-dependent sGC. Its hydrolysis is regulated by PDEs. Among at least seven isoforms of PDEs, PDE2, PDE5, and PDE9 are most prevalent in the brain. Figure 1 presents the main cGMP actions in the organism, and Fig. 2 depicts the NO/cGMP signaling pathway.

While our previous review [20] was on cGMP metabolism in the nervous system physiology, the present review is focused on alterations of the NO/cGMP signaling pathway in aging and Alzheimer's disease (AD).

NO/cGMP Signaling in Aging

Alterations in Nitric Oxide Synthase Expression and Activity

NO is produced by NOS enzymes [EC 1.14.13.39] which use L-arginine as substrate. These are complex proteins found constitutively in two isoforms: neuronal (nNOS) and

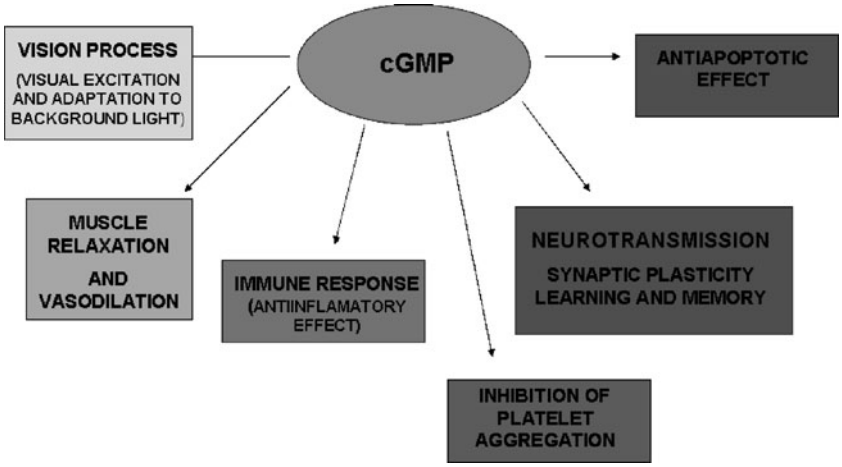

Fig. 1 cGMP functions in living organism. This secondary messenger has a major role in the vision process, vasodilation, muscle relaxation, inhibition of platelet aggregation, and neurotransmission. It also has an antiinflammatory and anti-apoptotic effect

endothelial (eNOS). The third isoform, (iNOS), is inducible type. iNOS is expressed in numerous cell types including macrophages and microglia and is induced under pathological conditions [26].

In 1997, McCann for the first time proposed the NO-dependent hypothesis of brain aging. According to this theory, repeated infections of central nervous system (CNS) and other organs may lead to the activation of iNOS in the brain. This mechanism may increase the concentration of NO, which together with disturbances in glutamate signaling, may cause the degeneration of neurons and glia. The main consequence of this process should be modification of hormone secretion and cognition in aging [41]. However, these changes are mainly connected with inflammatory processes that significantly modulate several molecular events during the life spans of humans and animals. Most of studies indicate that induction of iNOS does not occur during healthy physiological aging [10, 30, 34, 35].

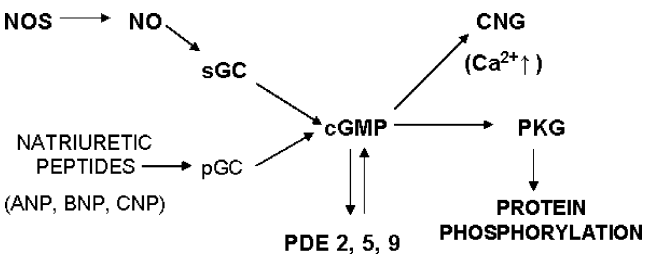

Fig. 2 NO/cGMP signaling scheme. Cyclic GMP (cGMP) can be synthesized via soluble guanylyl cyclase (sGC) activated by nitric oxide (NO), which is liberated by its synthase (nitric oxide synthase–NOS). The second possibility is cGMP synthesis via particular guanylyl cyclase (pGC) activated by natriuretic peptides (atrial–ANP, brain–BNP, and C type–CNP). In cells, cGMP has three main effectors—protein kinase G (PKG); cyclic nucleotide-gated ion channels (CNG), which can be opened after an increase in cGMP level; and phosphodiesterases (PDEs)—enzymes which hydrolyze phosphodiester bound in cyclic nucleotides

Considering the fact that sGC is the main NO receptor in the CNS that can activate cGMP synthesis at low nanomolar concentrations of NO [25], cGMP levels are affected by disturbances in NO synthesis under physio-pathological conditions.

Several findings indicate that NO synthesis is altered in the aged brain. Law et al. [33] observed high mRNA expression of the neuronal form of nNOS in several brain regions. Moreover, in their experiments cognitively impaired aged rats showed significantly higher nNOS mRNA expression than the cognitively unimpaired aged animals. However, several other studies indicated a lower mRNA level for nNOS in the senescent as compared with the mature brain [28, 30, 62]. However, results of [30] indicated concomitantly the enhancement of Ca2+ and calmodulin-dependent nNOS activity in all investigated brain parts with significantly higher value in the cerebellum. This study suggests that alteration of nNOS phosphorylation state may be responsible for the activation of NOS in aged brain. Moreover, the data suggest that the down-regulation of nNOS mRNA expression may be an adaptive mechanism that protects the brain against NO release.

The interpretation for nNOS gene suppression and its lower level of mRNA could suggest the activation of oxidative stress by NO, a subsequent alteration of NF–kB pathway and down-regulation of the gene as an adaptive mechanism. Liu et al. [34] and La Porta and Comolli [32] demonstrated also an increase in nNOS activity in the aged brain. In their last few studies, Liu et al. [34–36] support the contribution of NOS/NO to aging. Their data indicated a significant increase in the total NOS activity in aged dentate gyrus of hippocampus and in the other investigated brain parts as compared with younger rats with no change in nNOS protein levels. We have recently shown that nNOS plays a role in object recognition memory and that activation of this enzyme pool by inhibition of PDE2 is necessary for memory improvement in old animals [21].

Few studies have reported on eNOS expression and activity during aging. Available data indicated enhanced mRNA levels, no alteration in protein level and decreased activity [57]. Significant decrease in the eNOS protein level during aging was presented by Liu et al. [36] in CA2/CA3 layer of hippocampus but not across the dorsal and ventral hippocampus and no changes in the other investigated brain parts [34, 35]. However, the eNOS activity was not determined, and the alteration of protein level can only suggest lower eNOS activity. The changes in expression and activity of both constitutive NOS isoforms, eNOS and nNOS, have been implicated for the decline in cognitive functions in the senescent brain. eNOS plays an important role in regulation of vascular tone, vascular signaling, and remodeling, platelet aggregation, leukocyte–endothelial interactions, and also in angiogenesis [2]. NO synthesized by eNOS is the major endothelium-derived relaxing factor in the cerebral circulation [23]. In the CNS, NO, which acts on blood vessels, is produced by both eNOS and nNOS.

A third source of NO is iNOS, which can also be expressed in vascular cells in response to proinflammatory stimuli. Endothelial dysfunction in the brain can have various consequences since the eNOS-derived NO affects not only vascular muscle and blood elements, but also neuronal processes and neurogenesis [23].

A decrease in endothelial function and NO/cGMP signaling has been implicated in cognitive decline during aging. In the study by Mayhan et al. [40], it was shown that aging alters the eNOS-dependent reactivity of cerebral arterioles due to an increase in superoxide and oxidative stress. Within the vessel wall, reactive oxygen species may cause structural and functional changes that affect the regulation of cerebral perfusion and the permeability of the blood–brain barrier. These oxidative stress-induced alterations are thought to contribute to the initiation and progression of several cerebrovascular diseases [12]. A decrease in vascular NO synthesis activity can lead to a reduction of the protective effects of NO and increase in vascular wall inflammation, thrombosis, and smooth muscle cell proliferation. In general, it is important to recognize that the damage to endothelial cells and the reduction in vascular NO synthesis may contribute to alterations in the functioning of neuronal cells.

According to the results obtained by Chalimoniuk and Strosznajder in [10], the basal activity of NOS is increased while the level of cGMP is decreased in the aged brain comparing to brain of adult rats. However, in the presence of isobutylmethylxanthine (IBMX), a non-specific inhibitor of PDEs, the level of cGMP is higher in the aged brain than in the adult one. These results suggest that enhanced cGMP hydrolysis occurred during brain aging. Other studies provided evidence that the basal activity of NOS is decreased in the senescent brain in comparison to the mature one [43, 46, 58]. In addition, N-methyl-D-aspartic acid (NMDA)-dependent NO and cGMP synthesis was also decreased during the aging process [10, 58]. Discrepancies in alterations of NOS expression and activity during aging are probably be due to differences in environmental conditions, the level of oxidative stress, and in the methodology used in these studies.

Guanylyl Cyclase and cGMP Synthesis

After understanding the alteration in NO synthesis via different NOS isoforms during aging, the next step of the NO/cGMP pathway in the brain is sGC, so it is important to discuss whether its expression or activity is altered by the aging process. Because of the extensive role of the NO/cGMP signaling pathway in regulating brain function, alterations of sGC level may be a major factor contributing to age-related brain disorders. The study by Chalimoniuk and Strosznajder [10] has shown that, in the presence of IBMX, the nonselective PDEs inhibitor, cGMP levels are significantly higher in all parts of the aged as well as in the adult brains, suggesting that sGC is active in the senescent brain. Furthermore, incubation of mature and senescent brain slices with the highly selective PDE2 inhibitor, Bay 60-7550, resulted in similar increase in cGMP immunoreactivity [19]. On the other hand, there is evidence that the ability of cholinergic neurons in the basal forebrain and inter-neurons in the striatum to synthesize cGMP via the NO-dependent pathway was decreased during aging [22]. Study by Ibarra et al. [29] showed that, in the human cortex and striatum, sGC expression decreased with age. Vallebuona and Raiteri [58] found that the basal cGMP decreased in the hippocampus by 50% from 3 to 12 months of age, but remained constant with age in the cerebellum. NMDA-evoked cGMP response was higher in hippocampus of 3-month-old rats as compared to 12-month-old rats. Under this condition, similar cGMP response was impaired in cerebellum [10, 58]. However, the NO donor sodium nitroprusside (SNP) elicited cGMP responses were decreased slightly from 3 to 12–24 months in the hippocampus but not in the cerebellum. The non-specific PDE inhibitor IBMX produced large increases in hippocampal cGMP levels, and the age-related decrease in response was parallel to the decline in the basal level of cGMP. The NO/cGMP pathway has been implicated in neuron–glia interactions, synaptic plasticity, and long-term potentiation and depression [long-term potentiation (LTP) and LTD, respectively]. Taken together, these data indicate alterations in the cGMP turnover in senescent brain, with more active degradation than biosynthesis. Age-related decrease in cGMP level and its response to NMDA receptor might be an important causative factor underlying the cognitive decline in aging brain.

Consequences of Changes in NO/cGMP Pathway on Cognitive Performance of the Senescent Brain

The underlying mechanisms of cognitive impairment during aging have not been fully understood. Since activation of NMDA receptors leads to an increase of calcium in the post-synaptic neurons, several reports have implicated the role of the glutamate/NO/cGMP pathway in learning and memory [18, 37, 42]. There is further indication that learning and memory impairment in older animals is a consequence of reduced function of the glutamate/NO/cGMP pathway in the aged brain [47] and probably also alteration in the cholinergic signaling. The increase in PDE expression and activity may lead to more intensive cGMP hydrolysis in the senescent brain.

Recently, we have also shown the effects of inhibition of PDE2 and PDE5 on cognitive performance in 3-, 12-, and 24-month-old rats and on NOS activity in the hippocampus in animal's brains (Figs. 3 and 4). These results indicate that administration of the selective PDE2 inhibitor Bay 60-7550 could improve object memory in 3-, 12-, and 24-month-old rats and increase NOS activity in the hippocampus.

On the other hand, administration of a selective nNOS inhibitor together with Bay 60-7550 eliminated the memory improvement evoked by the PDE2 inhibitor.

Inhibition of PKG had no effect on NOS activity in the brains of old animals treated with nNOS and PDE2 inhibitors, suggesting that the increase of NOS activity after PDE2 inhibition is PKG-independent. The administration of the PDE5 inhibitor zaprinast had a positive effect only in adult animals [21]. It has to be mentioned that zaprinast is not selective for PDE5, as it also weakly inhibits PDE1, 9, 10, and 11 [5]; but, in the low concentration, we have used in our study it should not have any effect on PDEs other than PDE5.

These data suggest that inhibition of PDE2 could improve NO and cGMP-dependent cognitive performance in the aging brain.

Recent work by van der Staay et al. [59] revealed the positive effect of a new, highly potent PDE9 inhibitor, BAY 73-6691, on various aspects of learning and memory in young (2-month-old) rats. They also reported increased basal synaptic transmission as well as early LTP after weak tetanic stimulation in hippocampal slices from very old (31–35-month-old) rats. Taken together, these results indicate that agents inhibiting PDE9 seem to be an excellent in therapy for the cognitively impaired elderly, since this isoform is most selective for cGMP.

Eleven different phosphodiesterases PDE 1-11 families have been presented (also in our previous review [20]). These all enzymes are responsible for the regulation of second messenger's level cGMP and cAMP. Several from them are important in cognition, and also, under specific conditions, they are involved in neurodegeneration. In this short review, we have concentrated on PDE_s which are responsible for hydrolysis of cGMP. However, PDE 1 A,B, C, and PDE10, PD 11 that hydrolyze cGMP and cAMP and all four PDE4 subtypes that hydrolyzed cAMP, all these enzymes have gained recently increased attention as a potential new targets for cognition enhancement. The subject was described recently in review article by [52]. A few data should be shortly underlined that means presented by [53, 54] time-dependent involvement of cAMP and cGMP in consolidation of object recognition memory and improvement of long-term memory using selective inhibitors of PDE type 4. Moreover, Siuciak et al. [55, 56] described cognition and neurochemical alteration in mice deficient in PDE 4B and PDE 10A. Their recent data suggesting potential uses of PDE 4 and PDE 10A inhibitors in CNS disorders like schizophrenia and brain trauma. Little is known about the cognition enhancement after PDE-1 treatments. This PDE hydrolyses also cAMP and cGMP, and it seems that exerts positive effect on the brain function through regulation of blood brain circulation and metabolism [52].

NO/cGMP in Alzheimer Disease

In the CNS, NO at low physiological concentrations may act as an anti-apoptotic/prosurvival factor. However, at high concentrations and in the presence of superoxide radicals, NO can be toxic and can participate directly or through formation of peroxynitrite in protein nitration and nitrosylation, in lipid and DNA damage, and in neuronal cell death.

According to the last hypothesis, *S*-nitrosylation/redox signaling mediates protein misfolding and mitochondrial fragmentation in AD and also in Parkinson disease [11, 44]. Newly discovered evidence suggests that a number of proteins are *S*-nitrosylated. One such a molecule is protein-disulfide isomerase (PDI), an enzyme responsible for normal protein folding. The redox stress leads to SNO–PDI then to misfolded proteins and to neuronal cell death. Another protein that is *S*-nitrosylated in AD is the mitochondria fission protein–dynamin-related protein 1 [11].

Some of NO-evoked effects are mediated in part by cGMP and PKG [24]. For example, in the rat hippocampal neurons, the soluble form of the amyloid precursor protein (APP) could lower cytosolic calcium concentrations and improve neuronal survival against glutamate toxicity or glucose deprivation via the elevation of cGMP levels [4]. As shown in a number of studies using the selective sGC inhibitor ODQ (1H-[1,2,4]oxadiazole[4,3-a]quinoxalin-1-one) along with other NOS inhibitors, a basal level of

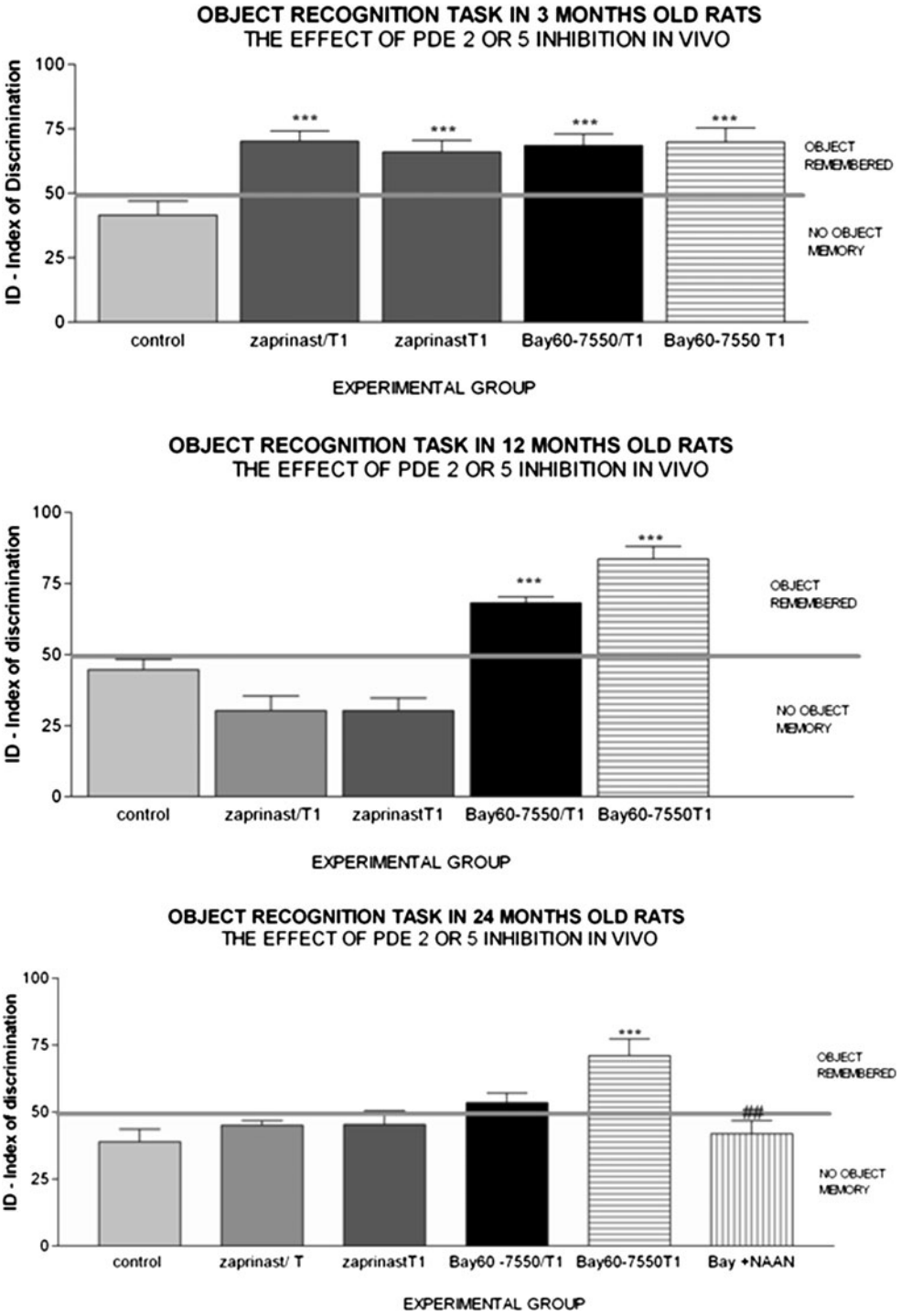

Fig. 3 Object recognition task after treatment with the PDE2 inhibitor Bayer 60-7550 (marked as Bay) or the PDE5 inhibitor zaprinast (marked as zap), in 3-, 12-, and 24-month-old animals. Inhibitors were injected subcutaneously 0.3 mg/kg 1 h before the first trial T1 (*name of inhibitor/T1*) or just after T1 (*name of inhibitorT1*). In the 24-month-old rats group, we also show the effect of subcutaneous administration of the nNOS inhibitor NAAN at a dose of 1 mg/kg of body weight together with the Bayer compound. They were administered 1 h before the beginning of the learning trial (*/T1*) of the object recognition task. The values are shown as mean±SEM of experiments with ten animals per inhibitor and time of injection. The statistical significance was evaluated by one-way ANOVA with Dunnett's post hoc test when the Bay 60-7550 compound was applied versus control (marked with *asterisk*, $p<0.0001$), and the effect of Bay 60-7550 was compared with the effect of Bay 60-7550 with the neuronal NOS inhibitor (NAAN) using a *t* test (marked as *number sign*, $p=0.005$; adapted from [21])

cGMP seems to be necessary for protecting cells against entry into the apoptotic pathway [13, 24]. Moreover, Wirtz-Brugger and Giovanni [60] have shown that cGMP also protects cells from death induced by nerve growth factor withdrawal and amyloid beta (A-beta). The data of Puzzo et al. [48] presented that AB peptides inhibits activation of NO/cGMP/cAMP-responsive element–binding protein (CREB) pathway during hippocampal synaptic plasticity. They suggest that enhancement of NO/cGMP signaling made provides a novel approach to the treatment of AD.

NO/cGMP pathway can suppress cell apoptosis by causing the phosphorylation of proapoptotic Bad protein, the inhibition of cytochrome c release and also the activation of caspase-3 through a PKG-dependent activation of the PI3 kinase/Akt kinase pathway [27]. The work

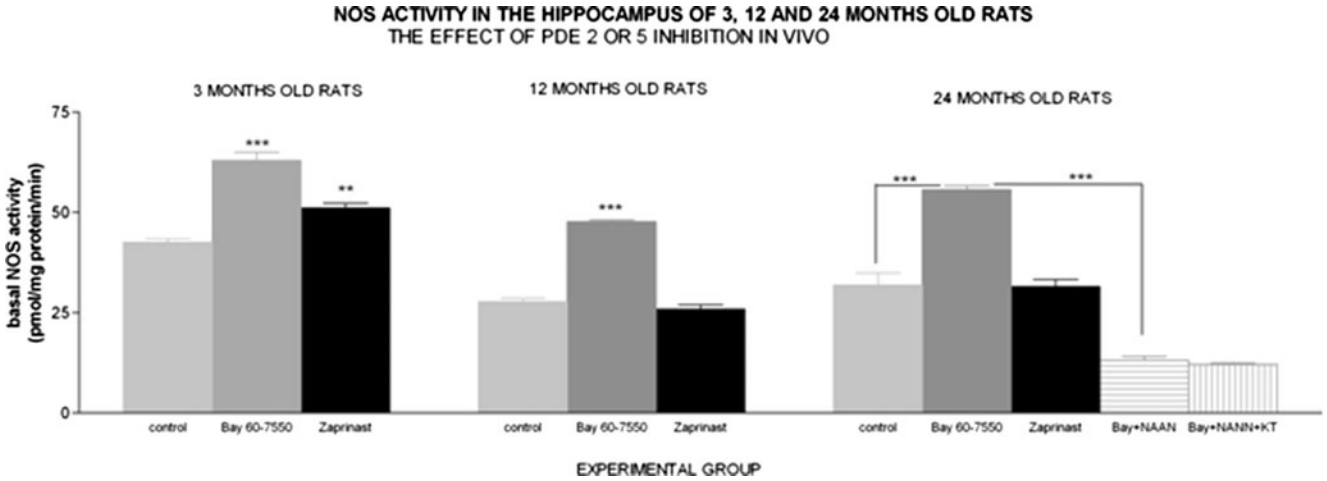

Fig. 4 NOS activity in the cytosol after in vivo administration of PDE2 or PDE5 inhibitor. Both compounds were administered subcutaneously at a dose of 0.3 mg/kg of body weight, dissolved in DMSO. Three independent experiments were carried out in triplicate, and the statistical significance was evaluated by a *t* test comparing inhibitor-treated animals to non-treated control animals ($p<0{,}005$). In 24-month-old rats, the nNOS inhibitor NAAN was also tested in vivo. Moreover, the PKG inhibitor KT5823 was tested in vitro (adapted from [21])

of Caretti et al. [8] indicated that, in hypoxia, PDE-5 inhibition by its selective inhibitor, sildenafil, reduces neuron apoptosis through an interaction involving ERK1/2 and p38. There is evidence that NO can exert opposite effects depending on the phase of apoptosis [6]. In the early phase, NO supports the survival of cells through a cGMP-dependent mechanism, but in the later phase, NO overproduction may contribute to the apoptotic process by reacting with excess superoxide anions, leading to peroxynitrite production and protein alterations by nitration and nitrosylation, mitochondria failure, and subsequently, neuronal degeneration and cell death.

All isoforms of NOS are altered in AD, indicating a critical role for NO in the pathology of this disease characterized by loss of neurons and synapses in the hippocampus and cerebral cortex and in certain subcortical regions. Accumulation of amyloid plaques and deposition of neurofibrillary tangles are the main features of AD. This degenerative disease AD has been identified as a protein misfolding disease (proteopathy) caused by abnormally folded A-beta and tau proteins in the brain. NO is a weak free radical. It can react with superoxide to generate peroxynitrite, a very toxic compound that easily penetrates membranes and can accelerate free radical cascade and damage mitochondria along with other subcellular organelles. The inflammation processes may additionally influence NO synthesis [16, 17]. Luth et al. [38] analyzed the expression of nNOS, iNOS, and eNOS in AD and control brains and compared the localization of NOS isoforms with the distribution of nitrotyrosine. In this study, nitrotyrosine was detected in neurons, astrocytes, and blood vessel cells in AD cases. Aberrant expression of nNOS in cortical pyramidal cells was highly co-localized with nitrotyrosine. Furthermore, iNOS and eNOS were highly expressed in astrocytes in AD, and double immunolabeling revealed that iNOS and eNOS are co-localized with nitrotyrosine in atrocytes. These studies suggest that increased expression of all NOS isoforms in astrocytes and neurons contribute to the synthesis of peroxynitrite and the generation of nitrotyrosine in proteins [38]. In the normal adult brain, while nNOS is the predominant form of NOS, some neuronal staining for eNOS was also observed in CA1 pyramidal neurons in the hippocampus. Immunoreactivity for iNOS was detected in the neurofibrillary tangle-bearing neurons in AD brain sections, but not in age-matched control sections. iNOS expression is associated with immune activation and thus is observed mainly in the reactive astrocytes and microglia surrounding A-beta deposits. eNOS immunoreactivity around affected blood vessels in AD patients was also observed. The data of Colton et al. [14] also support a role for nNOS neurons in AD. Results obtained by Yew et al. indicated a decrease in the number of NOS-positive neurons in the frontal cortex of AD patients and an increase of NOS staining in the AD hippocampus as compared to age-matched controls [42, 61]. Abe et al. [1] investigated the concentration of an endogenous NOS inhibitor, asymmetrical dimethylarginine (ADMA), in CSF from senescent and AD patients and found that AMDA concentration significantly decreased with age, whereas the arginine concentration was unaltered. ADMA levels were also decreased in AD as compared with age-matched controls. These findings suggest that ADMA may play an important role in regulating NO synthesis in brain aging and in pathogenesis of AD [1].

The soluble fragment of APP (sAPP) is shown to decrease $[Ca^{2+}]_i$ and has excitoprotective effect on target

neurons through increases in cGMP levels and activation of PKG [7]. Study by Barger et al. [4] indicated a significant increase in cGMP level within minutes of treatment of primary neural cultures with sAPP. Results obtained by Mattson et al. [39] indicated that the sAPP could enhance glutamate and glucose transport in synaptic compartments and that this effect is mediated by cGMP. In transgenic mice over-expressing human APP, 90% of A-beta plaques are associated with NOS-containing dystrophic neuritis, and nNOS activity was increased in the vicinity of A-beta plaques [50]. These results suggest that A-beta plaques induce neuritic dystrophy in cortical neurons containing NOS in this model of AD. Results further led to the hypothesis that toxic consequences of increased NO production might include subsequent degeneration of neurites and death of neurons as seen in the AD human brain [50]. Moreover, it was observed that neuronal death during AD is associated with increased expression of iNOS (mediated by TNFα) and is partially reversed by selective inhibitors of iNOS, which protect cells from the toxicity caused by microglial and monocytic secretory products [15].

Vascular damage and reactive gliosis are found associated with A-beta deposits in AD pathology. Freshly solubilized A-beta$_{1\text{-}40}$ was shown to enhance the vasoconstriction induced by endothelin-1 and increased resistance to relaxation triggered by NO [45]. These results suggest that A-beta may disturb the NO/cGMP pathway. Furthermore, these studies also showed that a cGMP hydrolyzing PDE inhibitor (dipyridamole) was able to block the enhanced vasoconstriction, as well as the resistance to relaxation induced by A-beta. cGMP, but not cAMP levels, are reduced after A-beta treatment of rat aortic rings. Moreover, A-beta induces a proinflammatory response in microglia, for example, leukotriene B4 release. Dipyramidole and other compounds that increase cGMP levels can prevent A-beta-induced microglial inflammation [45]. A-beta liberated from PC12 cells transfected with the human APP gene was shown to activate nNOS and iNOS [9, 31]. Subsequently, NO through cGMP-regulated PKG is involved in phosphorylation and activation of cytosolic phospholipase A_2 (PLA_2) and arachidonic acid release [9].

NO receptor and sGC activity have been shown to be affected by AD [29]. The activity of GC was investigated in the postmortem temporal cortex of AD patients [7]. sGC isoform showed a 50% decrease of activity after NO donor (SNP) treatment as compared with values obtained for control brains. The decrease in sGC expression in reactive astrocytes surrounding the A-beta plaques in AD brains could be the mechanism to prevent excess signaling via cGMP at sites of high NO production [3]. It is suggested that in AD, A-beta modulates hippocampal synaptic plasticity by down-regulating the NO/cGMP/PKG/CREB pathway [48]. In their last study, Puzzo et al. [49] report that PDE5-selective inhibitor sildenafil, through elevation of cGMP levels, is beneficial against the AD phenotype in a mouse model of amyloid deposition. After the treatment with sildenafil, authors observed an improvement of synaptic function, CREB phosphorylation, and animal's memory. These changes were associated with a long-lasting reduction of A-beta levels [49].

However, a study with AD patient's autopsy brains showed no change in PDE2 and PDE9 expression in AD brain compared with age-matched controls and that neither was PDE5 mRNA present [51].

General Summary

On the basis of all molecular events described above, it is reasonable to propose a hypothesis encompassing the NO/cGMP-dependent pathway in brain aging (Fig. 5). During the aging process, constitutive NOS activity is increased, which leads to increased NO production. This pool of NO could interact with the SH group of NMDA receptors leading to lower receptor response and NOS activation. At the same time, the activity of cGMP hydrolyzing PDEs is increased, cGMP levels are decreased, and the LTP process is less efficient in the senescent compared with the adult brain, resulting in worse cognitive performance of aged animals. By using a selective PDE2 inhibitor, we can restore cGMP levels, which induces a calcium influx trough

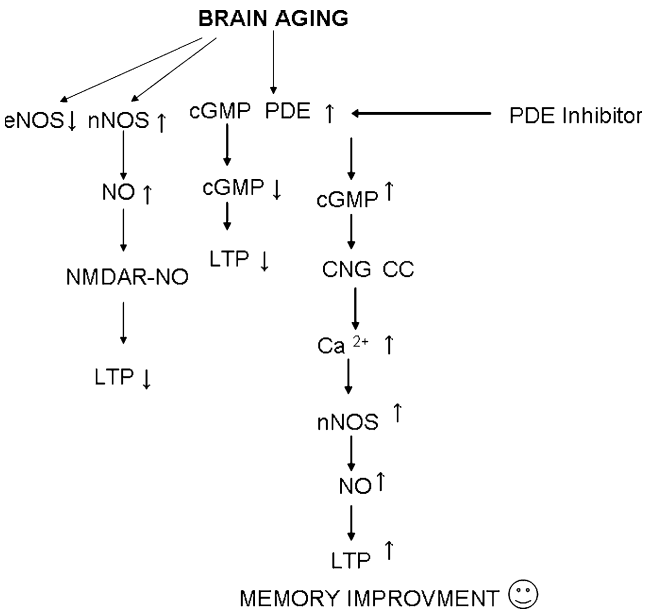

Fig. 5 NO/cGMP-dependent brain aging hypothesis. The following abbreviations are used in this figure: *cGMP* cyclic GMP, *CNG* cyclic nucleotide-gated ion (calcium) channel, *LTP* long-term potentiation, *NMDAR* NMDA receptor, *NMDAR-NO* NMDA receptor modified by NO, *NO* nitric oxide, *nNOS* neuronal isoform of nitric oxide synthase, *PDE* phosphodiesterase

cGMP-gated ion channels, activation of nNOS, and synthesis of NO pool engaged in improvement of cognitive performance during aging.

In contrast to AD, physiological aging does not lead to activation of iNOS. In AD, the increase in iNOS probably plays a key role in excessive NO production, and its interaction with superoxide radical evokes oxidative stress and damage of proteins, lipids, and finally, mitochondrial dysfunction. Oxidative stress also damages DNA, which leads to alteration of nuclear regulatory factors, activation of proapoptotic genes, and ultimately, cell death. However, preliminary data suggest no significant changes in cGMP levels in AD brain in comparison to age-matched controls.

In general, based on the above information, it is possible to conclude that new therapeutic strategies for the maintenance of a good cognitive performance in elderly people could be developed on the basis of cGMP metabolism. Selective agents that can enhance cGMP concentration and activation of PKG in the central and peripheral nervous system may be useful to protection against senile dementia.

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