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Amyloid β and neuromelanin—Toxic or protective molecules? The cellular context makes the difference

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Abstract

Alzheimer's disease (AD) and Parkinson's disease (PD) share several pathological mechanisms. The parallels between amyloid beta (Aβ) in AD and α-synuclein in PD have been discussed in several reports. However, studies of the last few years show that Aβ also shares several important characteristics with neuromelanin (NM), whose role in PD is emerging. First, both molecules accumulate with aging, the greatest risk factor for AD and PD. Second, in spite of their different structures, Aβ and NM have similar characteristics that could also lead to neuroprotection. Metals are required to catalyze their formation and they can bind large amounts of these metals, generating stable complexes and thus playing a protective role against metal toxicity. Moreover, they may be able to remove toxic species such as oligopeptides and excess cytosolic dopamine. Third, both Aβ and NM have been implicated in parallel aspects of the neuronal death that underlies AD and PD, respectively. For example, both molecules can activate microglia, inducing release of toxic factors such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and nitric oxide (NO). A careful analysis of these parallel effects of Aβ and NM, including their seemingly paradoxical ability to participate in both cell death and protection, may lead to an improved understanding of the roles of these molecules in neurodegeneration and also provide insights into possible parallels in the pathological mechanisms underlying AD and PD.

Keywords: Amyloid beta; Neuromelanin; Neurodegeneration

Contents

Abbreviations: AD, Alzheimer disease; Aβ, amyloid beta; APP, amyloid precursor protein; NM, neuromelanin; IL-6, interleukin-6; NO, nitric oxide; PD, Parkinson's disease; SN, substantia nigra; TNF-α, tumor necrosis factor-α; VMAT2, vesicular monoamine transporter 2

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1. Introduction

There is general interest among researchers in the commonalities between Alzheimer's disease (AD) and Parkinson's disease (PD), the two most common neurodegenerative disorders. Though AD and PD seem to be caused by different environmental and genetic factors, they appear to involve several similar pathophysiological events, including apoptosis, excitotoxicity, oxidative damage, toxic proteins, polymeric insoluble inclusions, and inflammation.

In PD a selective loss of dopaminergic neurons containing neuromelanin (NM) occurs, whereas the non-pigmented dopaminergic neurons are spared (Kastner et al., 1992; Gibb, 1992). NM appears as pigmented granules and is located in organelles that are often surrounded by a double membrane (Duffy and Tennyson, 1965). NM appears in early life and increases linearly thereafter (Fenichel and Bazelon, 1968; Zecca et al., 2002). The pigmented neurons of the substantia nigra (SN) and locus coeruleus have the highest levels of NM in the brain (Graham, 1979; Zecca et al., 2004a), and large amounts of iron are sequestered within the NM granules (Zecca et al., 1996). The NM is synthesized by oxidation of excess cytosolic catechols that are not accumulated in synaptic vesicles by vesicular monoamine transporter-2 (Sulzer et al., 2000). NM is a complex polymeric molecule whose structure is arranged as a multilayer system where each layer is a polymer composed of melanic groups bound to aliphatic and peptide chains (Zecca et al., 2003).

Amyloid β protein (Aβ) consists of 39–43 amino acid residues and is the main constituent of neuritic and diffuse plaques. It also is contained as cerebrovascular deposits that characterize the neuropathology of AD (Glenner and Wong, 1984; Kang et al., 1987). In AD, plaques develop in areas of the brain involved in memory and other cognitive functions (Arnold et al., 1991). Aβ is a cleavage product of a larger precursor protein, amyloid precursor protein (APP). Genetic studies together with the demonstration of Aβ neurotoxicity led to the development of the amyloid cascade hypothesis to explain the AD associated neurodegeneration process (Hardy and Higgins, 1992; Rozemuller et al., 1989; Selkoe, 2001). However, a modified version of this hypothesis has emerged that takes into account the fact that soluble oligomeric forms, protofibrils of Aβ and its intraneuronal accumulation also play a key role in the pathogensis of the disease (reviewed in Pereira et al., 2005).

A parallel can be drawn between Aβ and NM with respect to the relation of these two molecules to aging and metals on the one hand and neurodegenerative disease (AD or PD) on the other. Aβ and NM have different structures: Aβ (1–42) readily self-associates and forms nucleation centres, from which fibrils grow. The tendency of Aβ (1–42) to self-aggregate is the key reason for the scarcity of data on its fibril formation process.

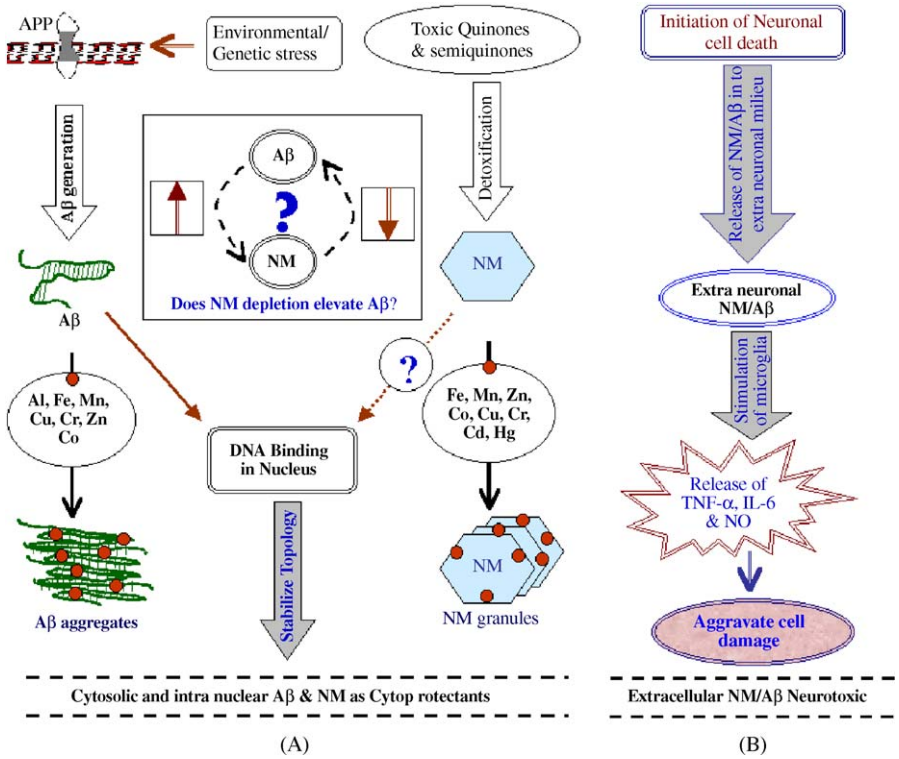

Fig. 1. Facts and opinions on different roles of NM and Aβ in physiological and pathological conditions. (A) The synthesis of NM is a detoxifying process removing excess cytosolic catechols. The cytosolic NM is widely viewed as playing a protective role by sequestering redox metals and other toxins. Aβ also binds different types of metals and it could be speculated whether Aβ plays a similar protective role in aging and AD. NM decreases in dopaminergic neurons in AD; this fact opens up a new thought, whether increased Aβ generation in AD is because of its ability to accumulate/chelate Cu, Zn and Al. (B) The extraneuronal Aβ/NM have been shown to activate microglia and stimulate the release of neurotoxic factors such as TNF-α, IL-6 and NO, further aggravating neuronal damage so that in such a context NM could be neurotoxic. An interesting issue is the reason for the presence of Aβ in the nucleus of apoptotic neurons in the hippocampus of the AD brain. Recent findings showing that Aβ binds and stabilizes DNA may partially explain this localization.

However, studies have shown that the internal structure of the Aβ fibril is a ladder of beta-sheet structure arranged in a cross-beta conformation (Stromer and Serpell, 2005). In contrast, NM is composed of dihydroxyindole–benzothiazine–peptide–aliphatic units arranged as a multilayer system. Despite these differences, these two molecules have several commonalities with respect to synthesis, accumulation in aging, affinity for metals, and apparent roles in both neuroprotection and neurotoxicity. In this review we will discuss parallels in the roles of Aβ and NM in the physiological and neurodegenerative processes that accompany AD and PD, as well as their roles in neurodegenerative disease more generally. Our hypotheses are summarized in Fig. 1.

2. Aβ and NM play an active role in neuroprotective processes by trapping metals and other potential toxins

Although NM levels are high in the dopaminergic neurons of the SN that degenerate in PD, it is not at all clear if this NM is part of the toxic events that underlie PD or a protective response that may slow the disease. In the SN of normal subjects, NM is proposed to be a neuroprotective compound due in part to the fact that it acts by trapping free radicals, and a wide variety of toxins (Zecca et al., 2003). In addition, NM appears to trap noxious redox active metals. For example, in dopaminergic neurons of SN the NM has been shown to scavenge Fe, as well as a variety of other reactive metals including Zn, Cu, Mn, Cr, Co, Hg, Pb, and Cd (Zecca et al., 2003, 1994, 1996; Bolzoni et al., 2002). In noradrenegric neurons of locus coeruleus NM also binds metal ions, including Fe and Cu (Zecca et al., 2004a).

Although the toxic amyloid hypothesis has dominated the study of AD for the past two decades, it is still unclear whether Aβ is sufficient or even related to the development of AD (Savory et al., 2002; Obrenovich et al., 2002). Indeed, it has been hypothesized that Aβ may actually be neuroprotective and, as will be discussed below, may even be a defensive consequence of an underlying disease mechanism (Joseph et al., 2001; Perry et al., 2000; Rottkamp et al., 2002; Obrenovich et al., 2002). As in the case of NM, Aβ peptides may have neuroprotective properties as a result of their ability to trap metals, as well as drugs, metabolites, and proteins (Atwood et al., 2003; Chan et al., 1999).

Thus an increase in Aβ generation in the AD brain and NM in the PD brain may slow disease progression. An added neuroprotective consequence may derive from the ability of Aβ and NM to form insoluble adducts. This may be followed by the eventual degradation of the adducts and consequent slow release of the toxins, which would in turn reduce the damaging effects that would occur if the toxin release was more rapid (Savory et al., 2002; Robinson and Bishop, 2002; Zecca et al., 2003).

The role of metal ions in neurodegenerative disease and the interaction between these ions and Aβ and NM deserve additional consideration. Metals have been implicated in neurodegenerative pathology in many studies (Rao et al., 1999; Doraiswamy and Finefrock, 2004; Zecca et al., 2004b, 2003; Bouras et al., 1997; Dexter et al., 1992; Bush and Strozyk, 2004; Oteiza et al., 2004; Carri et al., 2003; Beauchemin, 1998; Lovell et al., 1998). Metals have also been shown to potentiate Aβ aggregation in vitro (Bush et al., 1994; Fasman et al., 1995; Ricchelli et al., 2005), and this process would reduce the toxicity of both metals and soluble Aβ peptides (Savory et al., 2002). In dopaminergic cell cultures, Fe is required for NM synthesis (Sulzer et al., 2000), thus showing a further analogy between Aβ and NM. Studies carried out by Cherny et al. (2001) have shown that the metal chelator clioquinol can reduce Aβ plaques by solubilizing them.

Our own studies on progressive metal accumulation in the AD brain indicate that Fe accumulates in the early phase of AD, whereas Al is deposited preferentially only in the latter phase of the disorder (Rao et al., 1999). In PD, an early Fe accumulation in SN and an overload of Fe occurs in NM (Jellinger et al., 1992; Perl and Good, 1992; Bartzokis and Tishler, 2000). We hypothesize that NM also plays an antioxidant role in early AD trapping Fe, whereas in severe AD because of a decrease in NM in dopaminergic neurons (Reyes et al., 2003), Aβ could take up a parallel role because of its own ability to accumulate Al. The ability of NM to accumulate Al has also been reported (Zecca et al., 1994). In support of the above concepts, it has been well established that Al injection will enhance the Aβ deposition in aged rabbit animal model (Rao et al., 2000).

Another feature of Aβ and NM is their ability to bind to cholesterol and this, too, may be relevant to the possible neuroprotective actions of these two compounds. Some studies suggest that Aβ binds to cholesterol and lessens its adverse effects (Mori et al., 2001). Cholesterol has been shown to play a role in the pathogenesis of AD and, notably, serum cholesterol increases with advancing age, and diet-induced hypercholesterolemia enhances Aβ accumulation (Mori et al., 2001; Sparks et al., 1994). Similarly, it has been shown that NM also binds to cholesterol, and the implications of this are to be explored (Zecca et al., 2000).

Though the extracellular Aβ plaques have been implicated in AD, the intracellular presence of potentially amyloidogenic fragments of Aβ also is receiving attention. Moreover, it has been shown that Aβ is generated within intracellular compartments such as early endosomes, the trans-Golgi network, and dilated rough endoplasmic reticulum (Grant et al., 2000; Wild-Bode et al., 1997; Sparks, 1996). Aβ immunoreactivity was also found to be associated with microfilaments, outer mitochondrial membrane, and the nuclear envelope (Grant et al., 2000). Recently, we have shown that Aβ localizes in the nucleus of apoptotic neurons in AD hippocampus (Hegde et al., 2004). DeGiorgio et al. (2000) demonstrated co-localization of the Aβ and C-terminal domains of APP within the nuclei of SN pars reticulata neurons undergoing delayed degeneration.

The reason for the appearance of Aβ in nuclei of SN neurons is controversial. We think that the localization of Aβ at a subcellular level could play a very important role in sequestering toxic elements thereby protecting the DNA and proteins from oxidative damage, as does cytoplasmic NM in dopaminergic neurons of SN. We have found evidence for an altered conformation of genomic DNA (B → Z) in hippocampus of postmortem AD brain (Anitha et al., 2002) and also have

shown that Aβ stabilizes DNA topology by condensation (B → ψ) (Hegde et al., 2004).

3. Aβ and NM synthesis appear as a protective and compensatory response

As we have already suggested, whereas the toxic amyloid hypothesis has dominated the study of AD for the past two decades, it is still unclear whether Aβ is sufficient or even related to the development of AD (Savory et al., 2002; Obrenovich et al., 2002). Indeed, it has been hypothesized that Aβ may even serve as a defensive reaction to an underlying disease process (Joseph et al., 2001; Perry et al., 2000; Rottkamp et al., 2002; Obrenovich et al., 2002). Aβ has also been shown to have a neurotrophic effect at low (nM) concentrations. Although deposition of extracellular Aβ is an almost invariant event in the pathogenesis of AD, Aβ deposition is also observed in healthy aging brains (Price et al., 1991), and there is a lack of correlation between the amount of Aβ deposition and cognitive decline in AD (Terry, 1994; Berg et al., 1998). Moreover, in some of the APP and presenilin-I based transgenic mouse models of AD, the subtle functional deficits occur before the formation of Aβ plaques (Moechars et al., 1999). It also has been documented that oxidative stress precedes fibrillar depositions of Aβ (Misonou et al., 2000). Moreover, Aβ has been shown to be upregulated by many forms of stress, including injury and head trauma (Joseph et al., 2001; Misonou et al., 2000). It may be that Aβ is produced normally to bind neurotoxic solutes, such as metal ions, and precipitate into insoluble plaques. In short, Aβ formation may actually be a response to rather than a cause of neurotoxic oxidative challenges.

A similar debate has been running for a long time regarding the neuroprotective/neurodegenerative role of NM in PD (Zecca et al., 2003). It has been reported that NM synthesis is driven by excess cytosolic catechols that are not accumulated in synaptic vesicles (Sulzer et al., 2000). This conclusion, and in particular the importance of intravesicular inclusion of cathecols to the continued viability of catecholaminergic neurons, is supported by the evidence that in neuronal cultures over-expressing the vesicular monoamine transporter-2 (VMAT2), the formation of NM is strongly reduced (Zecca et al., 2003; Sulzer et al., 2000). Likewise, human dopaminergic neurons with lowest levels of VMAT2, such as the SN, have the highest NM content, whereas the VTA (Liang et al., 2004), which has a high level of VMAT2, has a low NM content. Therefore, NM synthesis appears to represent a protective/compensatory process that removes excess catechols that would otherwise produce peroxidation and neurodegeneration.

NM accumulates linearly in SN during aging; however in PD patients, NM levels drop to <50% of those in age-matched controls (Zecca et al., 2002; Fedorow et al., 2005). In locus coeruleus, NM concentration also increases linearly during aging with values similar to those found in SN (Zecca et al., 2004a), and is expected to decrease in PD as a consequence of norepinephrine neuron loss (Paulus and Jellinger, 1991; German et al., 1992). A decrease in the amount of histopathologically observable nigral NM in AD without nigral Lewy bodies also was found, and this was suggested as the consequence of retrograde degeneration from damage of nigral dopaminergic terminals in striatum by Aβ of the diffuse plaques (Reyes et al., 2003). Reduced NM content in dopaminergic neurons of SN, probably the consequence of lower dopamine concentration, is present in Rett syndrome, a progressive neurodevelopmental disorder with onset in early childhood, characterized by mental retardation, behavioral changes, movement disturbances, loss of speech, ataxia, apraxia, and other severe symptoms. Early loss of dopaminergic neurons and extraneuronal NM is observed in these patients (Jellinger, 2003). Whereas it is possible that the results simply indicate that the loss of dopaminergic neurons in SN is accompanied by the passive loss of their intracellular NM stores, it is also plausible that NM plays a causal role in that neurodegeneration. Such a hypothesis is supported by the neurotoxic effects of NM, which will be discussed in the next section.

In analogy with NM, it has been proposed that accumulation of Aβ as plaques in the AD brain and other diseases is a compensatory response to toxic species, particularly free radicals originating from the increased oxidative environment of the aged brain, inflammation, and head injury (Atwood et al., 2003; Shigenaga et al., 1994; Smith et al., 2000). As in the case of NM, neuronal degeneration can be induced by low molecular weight precursors of Aβ. Thus, the intraneuronal peptide Aβ (1–42), a precursor of Aβ, is neurotoxic, as is cytosolic dopamine, which is the precursor of NM (Wirths et al., 2004). APP processing occurs via several pathways that produce fragments with generally opposing properties, involving α-, β-, and γ-secretases (Estus et al., 1992; Smith-Swintosky et al., 1994; Furukawa et al., 1996). The understanding of the pathways for Aβ generation could throw light on its protective role. It is also important to determine if there is any other pathway for the peptide cleaved from APP.

H_2O_2 generation resulting from oxidative stress increases the concentration of both intracellular Aβ (McLean et al., 1999; Misonou et al., 2000) and secreted Aβ (Paola et al., 2000) in neuronal cell lines. Reactive oxygen species also increase Aβ production in human primary neuron cultures (LeBlanc, 1995; Berthon, 2000), suggesting that Aβ synthesis is influenced by stress conditions, possibly as a result of altered metabolism of metal ions such as Fe, Mn, Zn, and Cu. Indeed, Aβ burden has been shown to be inversely correlated with oxidative stress markers (Nunomura et al., 2001), suggesting that Aβ may have antioxidant effects (Cuajungco et al., 2000; Kontush, 2001). As we have already noted, a similar condition holds for NM, whose synthesis removes the excess cytosolic catechols, which would generate peroxidation processes and neurotoxicity. Again, analogous to Aβ synthesis, the NM synthesis also requires oxidative conditions generated by metals, enzymes, and other agents (Sulzer et al., 2000; Zecca et al., 1996).

4. Aβ and NM are also involved in toxic mechanisms

Although genetic data indicate a central role for Aβ in the etiology of AD (Kowalska, 2004; Steiner et al., 1999), the

molecular form associated with the neurodegenerative process has not been definitively identified. At present, the biological significance of the monomeric, misfolded isoforms, soluble β-oligomers, and the fibrillar aggregates is not fully understood. Indeed, for a variety of technical reasons, biophysical studies on the conformational diversity of different forms of Aβ and the conversion between the normal cellular form and the pathological conformations have failed to provide us with a clear picture of these events in AD. As already noted, the number of Aβ plaques do not always correlate with neurodegeneration or clinical dementia. Thus, a recent debate has focused on whether fibrillar (amyloid) or soluble oligomers of Aβ are active species of the peptide that ultimately causes the synaptic loss and dementia associated with AD (Cleary et al., 2005; Lansbury, 1999; Terry, 2001). Neurotoxicity of intracellular soluble Aβ oligomers precedes plaque (aggregate) neurotoxicity and also accounts for early stage memory loss (Cleary et al., 2005; Terry, 2001). Indeed, it is soluble Aβ levels and not plaque that are the best Aβ correlates of cognitive dysfunction in AD (Klein et al., 2001; Harper et al., 1999). In fact, the degree of dementia in AD correlates much better with Aβ assayed biochemically than with the histopathologically determined plaque counts (Naslund et al., 2000; Lue et al., 1999).

The relative impact of the soluble toxins may be particularly significant at the earliest stages of AD, including the transition from mild cognitive impairment to AD (Klein et al., 2001). Substantial evidence shows that Aβ-dependent toxicity can occur independently of extracellular plaque formation, and neurodegeneration precedes plaque formation, possibly involving intracellular Aβ accumulation (Sun and Chen, 2002; Wertkin et al., 1993; Wild-Bode et al., 1997). Recent studies support the toxicity of non-fibrillar oligomers and their possible causative role in neuropathology in AD, which is consistent with dissociation between fibril loads and the cognitive decline in AD (Cleary et al., 2005; Dickson and Yen, 1989; Terry et al., 1991; Lue et al., 1999).

There have been several studies and speculations on the properties and protective/toxic role of intraneuronal and extraneuronal NM in PD (e.g., Zecca et al., 2003). In SN of parkinsonian patients, excess Fe becomes associated with NM, and this iron is in a redox active state, in which it can actively participate in the formation of reactive oxygen species, as shown by several studies employing different methods (Jellinger et al., 1992; Zareba et al., 1995; Faucheux et al., 2003; Double et al., 2003; Galazka-Friedman et al., 2004). Proteasome inhibition was obtained by treating either dopaminergic neurons or the 26S proteasome fraction with NM, thus showing that proteasome inhibition can be induced by intraneuronal or extraneuronal NM (Shamoto-Nagai et al., 2004). Thus, in principle, NM could cause abnormal protein accumulation and neuronal death in PD. Aβ also inhibits the proteolytic activities of the 26S proteasome, which could, in turn, cause a marked decrease in Aβ(1–42) degradation (Lopez Salon et al., 2003). Proteasome inhibition by Aβ leads to a persistent exposure of the cells to the amyloidogenic peptides initiating neurodegeneration (Song and Jung, 2004).

The relationship between the protein aggregates and the mechanisms of cell dysfunction and death is controversial, and debates are in progress as to whether the protein aggregates constitute primary pathology, epiphenomena, or a protective response to another pathological process (Armstrong and Barker, 2001). Nonetheless, it seems likely that abnormal regulation of protein processing is a common feature of neuronal dysfunction and death in these two diseases (Trojanowski and Lee, 2000; Schulz and Dichgans, 1999). In brain affected by neurodegenerative disorders, abnormal aggregation of one protein is often associated with aggregation of other proteins.

α-Synuclein has been strongly implicated in the pathogenesis of PD (Goedert, 2001; Spillantini et al., 1997; reviewed in Hegde and Rao, 2003). Moreover, this protein, present in the lipid component associated with NM, has been found in those SN neurons that are more susceptible to degeneration in PD (Fasano et al., 2003; Halliday et al., 2005). This colocalization of α-synuclein and NM may facilitate the precipitation of α-synuclein and the consequent neuronal damage (Halliday et al., 2005). Recently, Lippa et al. (2005) showed that Aβ is also associated with α-synuclein aggregation in secondary Lewy body formation. Further, it had been shown that Aβ-40 plaque level is greater in cases with secondary α-synuclein aggregates (Lippa et al., 2005). However, Aβ(1–42) plaque level is not associated with α-synuclein aggregation (Lippa et al., 2005). Thus, Lippa and colleagues proposed that Aβ-40 plaque levels cannot be ruled out as a factor involved in secondary Lewy body formation. Nemes et al. (2004) reported that α-synuclein is associated with Alzheimer's neurofibrillary tangles. If the amyloid hypothesis can explain AD, can it also explain why some patients with AD develop PD-like symptoms? A very recent report showed that in mice the Aβ can cause dysfunction in extrapyramidal neurons in the striatum and the SN, two regions heavily affected in PD (Perez et al., 2005).

The NM released from dying neurons remains in the extra neuronal milieu and stimulates microglial release of neurotoxic factors like TNF-α, IL-6, and NO, potentially leading to a subsequent aggravation of neurodegeneration (Wilms et al., 2003). Extraneuronal NM was found in the brains of patients suffering from juvenile, idiopathic PD as well as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism, and a large number of activated microglia cells were observed in close vicinity to NM deposits (Ishikawa and Takahashi, 1998; Langston et al., 1999). The activation of microglia and the subsequent release of toxic molecules has also been reported for extracellular Aβ peptides (Monsonego et al., 2003; Ikezu et al., 2003). It appears that both Aβ and NM have a role in maintaining the neurodegenerative process via inflammatory mechanisms when liberated into the extracellular space once the neurodegenerative mechanism has been initiated by other processes.

5. What determines the balance between toxic and protective role of Aβ and NM?

We hypothesize that the main conditions that switch the behavior of Aβ and NM from neuroprotection into neurotoxi-

city are leaking or breaking of membranes, toxin overload, ongoing neurodegenerative processes, and inadequate cellular localization. When a genetic or environmental factor initiates neuron degeneration with the breaking of the neuronal membrane, the released NM activates microglia (Langston et al., 1999; Wilms et al., 2003). This, in turn, produces neurotoxic compounds, all of which cause neuronal death with the further release of NM and microglia activation and establishes a chronic neurodegenerative process.

Toxic leakage of NM could also occur within the cell. For example, if an increased permeability of the membrane of the NM organelle occurs, the NM could be released and interact with cytosol, causing inhibition of 26S proteasome with consequent abnormal accumulation of misfolded/aggregated proteins. Overload of metals, like iron, generates labile NM–Fe adducts with high reactive Fe, which can produce the Fenton reaction, induce protein aggregation, and interact with active sites of enzymes (Zareba et al., 1995; Double et al., 2003; Faucheux et al., 2003; Ostrerova-Golts et al., 2000).

Likewise, even though fibrillar Aβ deposition occurs predominantly extracellularly in AD, the initiation of neuronal death releases more Aβ, which will activate microglia leading to aggravation of the neurodegenerative process. Reactive microglia plays an important role in the pathogenesis of AD (Bodles and Barger, 2005). The enhancement of secreted Aβ results in the activation of microglia and the generation of oxygen radicals, and damages membrane lipids and other proteins. Aβ production has been shown to be stimulated under oxidative stress stimuli (Mattson and Goodman, 1995).

As a part of its neuroprotective role, NM accumulates a large amount of redox active metals (Fe, Cu, Mn) and non-redox toxic metals (Cd, Hg) and organic toxins, like pesticides. However, the extraneuronal degradation of NM by microglia and intraneuronal degradation can suddenly release high concentrations of these toxins. Aβ also traps redox metals like Cu, Fe and Zn (Savory et al., 2002). However, it has been shown that unbound redox metals favor Aβ toxicity (Rottkamp et al., 2001). Soluble Aβ has been shown to be more toxic to the cells than the fibrillar forms. Klein et al. (2004) reported that small assemblies of unmodified Aβ were the proximate neurotoxin in AD. El-Agnaf et al. (2000) showed that the oligomeric form of Aβ was more toxic. Thus, the current debate is on what forms of Aβ are neurotoxic. Recently, Lee et al. (2004) challenged the amyloid cascade hypothesis and suggested senile plaques and Aβ could be a protective adaptation to AD. However, unlike the NM, Aβ deposits are resistant to degradation.

Ongoing neurodegenerative processes can damage membranes thus exposing NM and Aβ to abnormal interactions and involving these molecules in pathologic pathways as mentioned above. Regarding cellular localization, the extracellular occurrence of NM is always associated with a pathological activation of microglia, while intracellular NM is normally involved in physiological/neuroprotective pathways.

The cellular localization of Aβ is a more complex phenomenon. Though the principal Aβ deposits are found extraneuronally, recent studies point to the importance of intraneuronal and intranuclear Aβ. However, neither the time course of the appearance of intraneuronal Aβ in AD nor its contribution to the neurodegenerative process is clearly understood (Gouras et al., 2005). However, it appears that initially the Aβ is generated in intraneuronal mileu as a cytoprotective strategy to trap or immobilize redox metals and other oxidative species (Savory et al., 2002). However, as the disease progresses due to accumulation of more Aβ–metal complexes that fibrillize faster than normal Aβ, the Aβ–metal fibrils will disrupt the normal neuron functioning.

6. Conclusions and implications

Aβ and NM are molecules with different structures but many common features in brain aging and disease. Both molecules accumulate in brain regions targeted by AD and PD during aging. Whereas only the neurotoxic effects of Aβ and NM have been emphasized in the past, during the last few years, experimental evidence showing a protective/compensatory role has been put forth. For example, Aβ and NM can bind toxic metals such as Fe, Al, and Cu, forming a stable adduct, thus protecting brain cells. Other toxic compounds are efficiently immobilized, as well. Furthermore, the synthesis of Aβ and NM, respectively, removes toxic peptides and excess cytosolic dopamine, again playing a neuroprotective role. On the other hand, it also appears these molecules have toxic properties, mostly as a result of the induction of inflammatory responses. Therefore, these two molecules have intrinsic properties that may be differentially expressed depending on the cellular context, so that the same molecule can enter into a physiological or pathological process and should not be considered prejudicially toxic or protective, such as in the past when Aβ and NM were considered to be only toxic.

Considering the key physiological/compensatory role played by NM, pharmacological interventions aimed to either inhibit NM synthesis or induce degradation of intraneuronal deposits of NM would also block important neuroprotective mechanisms. In fact, defects of NM synthesis are often associated with disease states. The aggregation of α-synuclein and tau-protein also appears as compensatory processes that should not be pharmacologically blocked. On the other hand, drugs able to remove extraneuronal deposits of NM, typically present in parkinsonian SN, could be effective in slowing down the progression of PD. Chelating agents able to specifically remove the toxic metals bound to NM could help to unload such a toxin reservoir, which could suddenly leak and damage the neuron when NM organelle is degraded.

Overexpression of Aβ has been considered to be associated with the disease. We think that once Aβ is overproduced, the transition from the toxic intracellular soluble forms to the less toxic aggregated forms has been a compensatory measure adopted by the cell to reduce the toxicity of Aβ. Therefore, pharmacological treatments with the purpose of inhibiting/disrupting the aggregated form would aggravate the disease conditions. The therapeutic interventions should aim at the inhibition of its over-expression and stimuli that trigger Aβ over-expression. Chelating agents are applicable in AD as there

has been an overload of metal accumulation, which can prevent the metal induced toxicity and also overproduction of Aβ.

Considering the large variations in brain content and form of Aβ and NM in disease, these two molecules could be used as markers to be detected by developing adequate in vivo imaging methods for early diagnosis and monitoring of disease progression in AD and PD (Zecca et al., 2005).

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