Neuromelanin of the substantia nigra: a neuronal black hole with protective and toxic characteristics

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Neuromelanin accumulates in dopaminergic neurons during normal aging, and in Parkinson's disease, neurons with this pigment are those that selectively degenerate. Intraneuronal neuromelanin could play a protective role during its synthesis by preventing the toxic accumulation of cytosolic catechol derivatives and, in addition, by its ability to scavenge reactive metals, pesticides and other toxins to form stable adducts. However, dying neurons in Parkinson's disease that release neuromelanin might induce a vicious cycle of chronic neuroinflammation and neuronal loss.

The most highly pigmented cells in the human brain are the dopaminergic neurons of the substantia nigra and the noradrenergic neurons of the locus coeruleus [1,2]. The pigment, which is present in primates including chimpanzee, gibbon and baboon (and in their more distant relatives, such as horse and sheep [3,4]), is composed of neuromelanin (NM). This electron-dense substance is located in organelles surrounded by a double membrane in the neuronal perikaryon [5] that are known as NM granules. Parkinson's disease (PD) is characterized by preferential loss of those dopaminergic neurons that contain NM [6,7]. Thus, it is important to identify the role of NM in the substantia nigra in physiological conditions and in the pathogenesis of PD. This article reviews studies from the past ten years on the molecular aspects of NM, and discusses these structural aspects in the light of brain aging and PD.

Neuromelanin structure

Neuromelanin is a complex polymeric molecule that has a multilayer (graphite-like) 3D structure similar to the melanins (eumelanins) in hair, feathers and skin (R. Crippa *et al.*, unpublished), with planar overlapped sheets consisting of dihydroxyindole and benzothiazine rings and additional unidentified groups [8,9]. These sheets are stacked much higher in NM than in other synthetic or naturally occurring melanins (R. Crippa *et al.*, unpublished). A consistent aliphatic component with an as-yet unknown structure is a distinctive characteristic of NM not found in other melanins [10–12]. Neuromelanin contains both Fe^{3+} and quinone-like radicals with characteristic peaks measured by electron-spin resonance [13]. Approximately 20% of the mass of NM granules in human substantia nigra consists of a novel class of polyunsaturated lipids with high molecular mass, low volatility and low oxygen content [10]. The 'aging pigment', lipofuscin, is also a component of human NM organelles. Neuromelanin might participate in the synthesis of these lipids, or the lipids could be derived as breakdown products during an autophagic pathway.

Synthesis of NM

It has long been debated whether NM synthesis is due to enzymatic oxidation or auto-oxidation of dopamine. The involvement of tyrosinase, which catalyzes conversion of tyrosine to L-dopa and to dopa-quinone in eumelanin synthesis, has been proposed by some authors [14,15] but denied by others [16]. Albinos that lack tyrosinase, however, have a normally pigmented substantia nigra [17]. Alternative enzymatic pathways have been suggested for NM synthesis, including catalysis of dopamine-quinone synthesis by tyrosine hydroxylase, prostaglandin H synthase, peroxidase, macrophage migration-inhibitory factor and other enzymes [18,19]. Neuromelanin could, alternatively, be derived from non-enzymatic oxidation, and auto-oxidation of catecholamines to quinones with addition of a thiol group has been demonstrated in the brain [20]. The synthetic compound dopamine-melanin can be synthesized by the auto-oxidation of dopamine, although such synthetic melanin compounds have important structural differences from NM [10,13]. Recently, NM synthesis was experimentally induced in cultured rat substantia nigra neurons exposed to L-dopa [21]. This model reproduced nigral NM in that the chemical structure as measured by electron-spin resonance was identical to human NM, and the pigment was localized in the characteristic organelles surrounded by double membrane. These organelles were apparently autophagic vacuoles that arise as a response to cellular stress and sequester cytosol and damaged organelles for transport to lysosomes for degradation. The formation of NM in culture was blocked by iron chelation and by overexpression of the vesicular monoamine transporter, VMAT2. Thus, NM synthesis appears to be driven by an excess of cytosolic catecholamines that are not accumulated in synaptic vesicles (Figure 1). It might be that excess cytosolic dopamine produces dopamine-quinone in the cytosol that reacts with proteins and lipids to cause peroxidation and neuronal damage. Thus, it appears that NM biosynthesis is a protective process in which damaged organelles, proteins and lipids, perhaps including dopamine-glutathione, are targeted for lysosomal degradation. It also appears that NM

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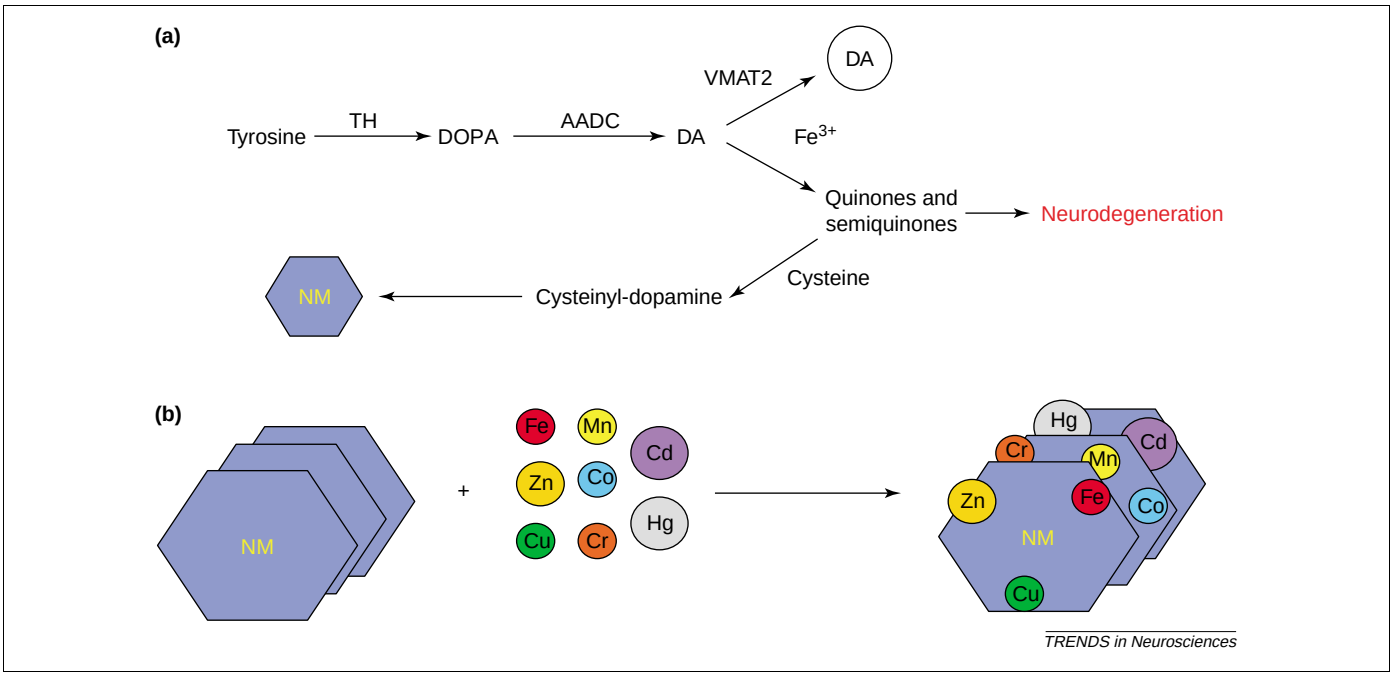

Figure 1. The protective role of neuromelanin. **(a)** The synthesis of neuromelanin (NM) removes excess cytosolic catecholamines that are not accumulated by synaptic vesicles, thus preventing the consequent damage. In this example dopamine (DA), synthesized from tyrosine by tyrosine hydroxylase (TH) and aromatic amino acid decarboxylase (AADC), is normally taken up into vesicles by the vesicular monoamine transporter VMAT2. Excess DA can interact with Fe^{3+} to form quinones and semiquinones. If these react with cysteine to form cysteinyl-dopamine, which can be further converted into neuromelanin, neurodegeneration can be avoided. **(b)** Exogenous metals can be released in reactive and potentially toxic forms in the cytosol; toxic metals such as Cd and Hg can also reach the neuronal cytosol following environmental exposure. Neuromelanin chelates toxic metals in the cytosol, forming stable complexes. Such chelation blocks toxic effect of metals, including Fenton's reaction and enzyme inactivation. Abbreviation: DOPA, dihydroxyphenylalanine.

autophagic vacuoles undergo very slow or interrupted in lysosomal fusion, so that the pigment accumulates over normal aging.

Interaction of NM with toxins and metals

MPP + , a metabolite of the parkinsongenic toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), binds to NM, and so NM could reduce the toxicity of MPP + [22]. The herbicide paraquat, also a potential PD-inducing agent, is accumulated by NM in neurons, which might likewise be protected against toxicity [23]. Neuromelanin also binds chlorpromazine, haloperidol and imipramine, modulating their effects and toxicity [24]. The dihydroxyindole groups present in NM are responsible for the strong chelating ability of NM [13]. Neuromelanin binds to a variety of metals, including Zn, Cu, Mn, Cr, Co, Hg, Pb, Cd and, in particular, Fe [13,25]. The level of nigral Fe increases by 30–35% in PD, owing to accumulation within NM granules [26,27]. Ferric iron in NM is bound by oxygen-derived phenol groups so that NM sequesters redox-active Fe ions, reducing the formation of hydroxyradicals [28]. This bound Fe exists as a high-spin complex with octahedral configuration [13] and the Fe sites are arranged in a ferritin-like ironoxyhydroxyde-cluster form [29,30]. The ability of NM to act as a 'black hole' capable of chelating redox-active metals [13,25] and a wide variety of drugs suggests that it could be a high capacity storage trapping system, and as such might prevent neuronal damage (Figure 1). It has also been suggested that the accumulation of toxic compounds by NM might be followed by a slow release of the toxin. However, this is unlikely because we have observed a high storage capacity of NM for toxic metals and have never found a saturation of this capacity in NM of human substantia nigra [13,25,31].

Neuromelanin during aging and PD

Neuromelanin accumulates normally with age in human, rat, canine and primate substantiae nigrae [32]. In humans, the first NM granules appear around the third year of life [33] and NM concentrations in the substantia nigra increase linearly over aging, reaching values as high as 4.0 mg g^{-1} in those aged in their eighties. By contrast, in PD patients, NM concentrations drop to $<50\%$ of those in age-matched controls [34]. In subjects with idiopathic PD, MPTP intoxication and some parkinsonian syndromes, insoluble extraneuronal NM granules arise following substantia nigra cell death and remain in the extracellular milieu in large amounts for long periods. The extraneuronal NM is phagocytosed by microglia and is associated with microglial activation [35,36]. A recent study demonstrates that NM in microglia cultures induces chemotaxis and stimulates release of the neurotoxic mediators tumor-necrosis factor α (TNF-α), interleukin 6 (IL-6) and nitric oxide (NO). Treatment with sulfasalazine, which inactivates nuclear factor (NF)-κB, or with SB203580, which inactivates p38 mitogen-activated protein kinase (MAPK), inhibits microglia activation and release of TNF-α, IL-6 and NO [37]. Thus, in PD, although different mechanisms such as environmental toxins and genetic factors can initiate neuronal damage in the substantia nigra and striatum, NM released from dying neurons induces release of neurotoxic microglial factors, potentially leading to a subsequent aggravation of neurodegeneration (Figure 2). This activation of microglia by release of toxic molecules has also been reported for extracellular α-synuclein (H. Wilms *et al.*, unpublished). In idiopathic PD, the neurons are depleted in both the substantia nigra and locus coeruleus, whereas in MPTP-intoxicated subjects, locus coeruleus neurons are spared

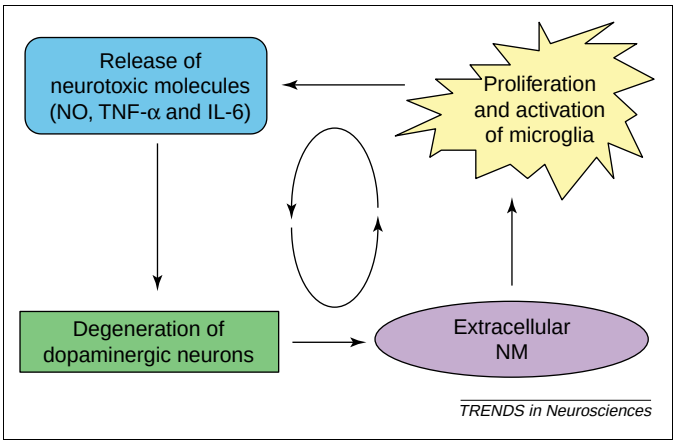

Figure 2. The toxic role of neuromelanin. Following a neuronal damage (due to an environmental or genetic factor), the released neuromelanin (NM) induces microglial activation, with production of the neurotoxic molecules tumor-necrosis factor α (TNF-α), interleukin 6 (IL-6) and nitric oxide (NO), which damage other neurons. These degenerating neurons will release NM, then establishing a chronic condition of neuroinflammation and neurodegeneration.

[36]. This different vulnerability might be explained in part by structural differences in NM of substantia nigra and locus coeruleus. In any case, extraneuronal NM is a strong candidate to be a cause of chronic inflammation and cell death in the substantia nigra in PD.

Future studies should point to the characterization and role of NM in brain regions, including the locus coeruleus and others that are targeted by PD, because there might be important differences in the structure, synthesis and function of NM in these regions. Moreover the precursors and biochemical steps involved in NM synthesis need to be identified to further elucidate the normal cellular role of this pigment.

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