Substantia nigra neuromelanin: structure, synthesis, and molecular behaviour

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Abstract

The pigmented neurones of the substantia nigra are typically lost in Parkinson's disease; however, the possible relation between neuronal vulnerability and the presence of neuromelanin has not been elucidated. Early histological studies revealed the presence of increasing amounts of neuromelanin in the substantia nigra with aging in higher mammals, showed that the neuromelanin granules are surrounded by a membrane, and comparatively evaluated the pigmentation of the substantia nigra in different animal species. Histochemical studies showed the association of neuromelanin with lipofuscins. However, systematic investigations of the structure, synthesis, and molecular interactions of neuromelanin have been undertaken only during the past decade. In these later studies, neuromelanin was identified as a genuine melanin with a strong chelating ability for iron and an affinity for compounds such as lipids, pesticides, and MPP[+]. The affinity of neuromelanin for a variety of inorganic and organic toxins is consistent with a postulated protective function for neuromelanin. Moreover, the neuronal accumulation of neuromelanin during aging and the link between its synthesis and a high cytosolic concentration of catechols suggest a protective role. However, its putative neuroprotective effects could be quenched in conditions of toxin overload.
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Black/brown pigmented granules in the human central nervous system has been observed since the 1930s.[1] The most pigmented regions are two mesencephalic areas: Sömmering's substantia nigra and the locus coeruleus.[2–4] Histological studies displayed pigmentation in the substantia nigra of other mammals phylogenetically close to humans, including the chimpanzee, gibbon, and baboon, and more distant ones, such as horses and sheep.[5–7] Histochemical studies on human substantia nigra and locus coeruleus found that the pigment had similar properties to the melanins,[8 9] including being insoluble in organic solvents, being bleached by hydrogen peroxide, and being labelled by silver stains.[10] The pigment was therefore named neuromelanin.

Histological studies showed that neuromelanin granules were located in the neuronal perikaryon and were surrounded by a double membrane.[11–13] In humans and horses, histochemical analyses indicated an association of neuromelanin granules with lipofuscin.[14 15] In the substantia nigra, neuromelanin accumulates during aging[2 3 16 17] and is found after the first 2 to 3 years of life.[18]

Parkinson's disease is a neurodegenerative disorder caused by the selective death of pigmented substantia nigra neurones,[19 20] giving rise to dopamine depletion in the neostriatum,[21 22] and resulting in a clinical syndrome characterised by tremor, rigidity, and severely impaired motility. The pigmented substantia nigra neurones are more vulnerable than the non-pigmented ones.[2] However, important questions remain regarding the possible role of neuromelanin in the substantia nigra, both under physiological conditions and in the pathogenesis of Parkinson's disease. Here, we review those studies undertaken during the past 10 years on the molecular aspects of neuromelanin, and attempt to integrate these structural aspects with morphological findings.

Structure

Initially, the name neuromelanin was chosen because of its similarity in appearance to cutaneous melanin. However, recent electron paramagnetic resonance (EPR) and metal analysis studies indicate that chemically neuromelanin is indeed a genuine melanin because it has a stable free radical structure and avidly chelates metals.[4 23–25] The ability of neuromelanin to interact with several inorganic and organic compounds, including metal ions and lipids, complicates studies of the structure of this pigment.

Degradation analyses using potassium permanganate and hydriodic acid hydrolysis showed that neuromelanin has properties of both pheomelanins and eumelanins.[26 27] Elemental analyses of neuromelanin revealed a high sulphur content (2.5–2.8%), with a molar C/H ratio lower than that of synthetic melanins,[27–29] thus indicating the presence of aliphatic groups and benzothiazine rings. Infrared spectroscopy of neuromelanin revealed the presence of aliphatic groups and a low intensity aromatic component, whereas in synthetic melanins the aliphatic groups were absent.[28–30] Chemical degradation studies showed that neuromelanin contains equal amounts of indole and benzothiazine molecules.[27]

Neuromelanin consistently shows a peptide component of about 15%.[31] The amino acids could be derived from a direct reaction between the melanic polymer and proteins,[31] or dopamine molecules bound to cysteinic residues of polypeptidic chains. Indeed, the

precursor of neuromelanin synthesis has been suggested to be cysteinyl-dopamine,[26 28 32 33] although a study using hydriodic acid hydrolysis failed to identify the corresponding degradation products.[34] Moreover, nuclear magnetic resonance spectroscopy indicates that the presence of both aliphatic and aromatic hydrogens, and the ratio of aliphatic to aromatic hydrogens is again higher in neuromelanin than in synthetic melanins,[30] suggesting that dopamine cannot be the only precursor in neuromelanin synthesis.

x Ray diffraction studies have shown that neuromelanin has a multilayer (graphite-like) three dimensional structure similar to synthetic and naturally occurring melanins.[35 36] The three dimensional structure is derived from planar overlapped sheets consisting of cyclic molecules of indolebenzothiazine rings. However, these sheets are stacked much higher in neuromelanin than in any other synthetic and naturally occurring melanins.[36]

Biosynthesis

The process of neuromelanin formation is obscure, although a recent in vitro study has clearly established some steps of this complex process.[37] It has long been debated whether the synthesis of neuromelanin is enzymatically mediated or whether it is a pure autoxidation process of dopamine derivatives. For eumelanin synthesis, the enzyme tyrosinase (also known as monophenol monoxygenase) catalyses the conversion of tyrosine to L-dopa and then to dopa-quinone.[38] Some authors proposed that tyrosinase could also be involved in neuromelanin biosynthesis because tyrosinase mRNA[39] and promoter activity[40] have been detected in the substantia nigra. However, tyrosinase has not been detected in the substantia nigra by immunohistochemistry.[41] Moreover, albinos who lack tyrosinase display normally pigmented substantia nigra.[10]

Alternative enzymatic actions have been suggested, including tyrosine hydroxylase mediated oxidation of dopamine.[42] In another study, peroxidase catalysed the oxidation of tyrosine to dopa and then dopamine, and further oxidisation to the respective quinones that are possible precursors of neuromelanin.[43] It was proposed that prostaglandin H synthase, which has peroxidase activity and is located on the mitochondrial membrane, could mediate the oxidation of dopamine to dopamine-quinone, which can internally cyclise and, by the addition of the amine group on the aromatic ring, form an indole derivative called dopaminochrome.[44 45] In addition, enzymatic activity of macrophage migration inhibitory factor was suggested for neuromelanin synthesis, because it converts catecholamines into dihydroxyindole derivatives, which are potential precursors of neuromelanin.[46]

Alternatively, neuromelanin could derive from non-enzymatic oxidation. The autooxidation of catechols to quinones with the addition of a thiol has been demonstrated in the brain.[47] A dopamine-melanin can be synthesised by the autooxidation of dopamine, although there are several structural differences between synthetic melanins and the natural one isolated from the substantia nigra.[4 29–31] Recently, neuromelanin synthesis was induced in rat substantia nigra neurones and PC12 cell cultures by exposure to L-dopa.[37] The pigment produced in this model contains a stable free radical; in addition, both light and electron microscopy have shown that the pigment synthesised in these cells appears to be identical to human neuromelanin, and the granules are surrounded by a double membrane, similar to the naturally occurring neuromelanin of the substantia nigra.[37] In those experiments, treatment with the iron chelator desferrioxamine inhibited neuromelanin synthesis stimulated by L-dopa; therefore, it seems that iron is involved in neuromelanin formation. In this model, neuromelanin synthesis was shown to be driven by an excess of cytosolic catecholamines not accumulated in synaptic vesicles.

Interaction of neuromelanin with organic compounds

Neuromelanin interacts with numerous organic molecules including lipids, pesticides, and toxic compounds. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxin that after systemic administration selectively kills substantia nigra neurones by conversion through monoamino-oxidase type B activity to methylphenylpyridine (MPP^+), which in turn stops the respiratory chain at the NADH-CoQ1 reductase stage,[48–50] leading in humans and several other animal species to an irreversible parkinsonian syndrome.[51 52] Neuromelanin might reduce the toxicity of MPTP by accumulating its toxic metabolite MPP^+ in vivo.[53]

The herbicide paraquat has a molecular structure similar to that of MPTP, and has been proposed as a Parkinson's disease inducing agent. The pesticide is accumulated in neuromelanin containing nerve cells, where it appeared that the neuromelanin adsorbed intraneuronal paraquat, protecting the neurones from consequent damage.[54]

Neuromelanin can also accumulate chlorpromazine, haloperidol, and imipramine, thereby contributing to the control of the intraneuronal concentration of these molecules.[55] Because higher intraneuronal concentrations of dopaminergic drugs might be toxic to substantia nigra neurones, neuromelanin can influence this toxicity.

The association of neuromelanin with lipids has been described in several studies.[28 31 56] Although previous studies proposed that lipids were part of the neuromelanin molecule, recent work has shown that neuromelanin contains about 20% adsorbed lipids.[31] Cholesterol is a minor component in this lipid mixture, with the major component being a new class of polyunsaturated lipid with a high molecular mass, low volatility, and low oxygen content.[31] It may be that neuromelanin itself catalyses the synthesis of this type of lipid. Alternatively, neuromelanin could originate from lipofuscin by an enzymatic reaction occurring in lysosomes,[11 14] although this hypothesis is not supported by recent observations.[37 57] In this case,

high molecular mass lipids could be derived from a lysosomal metabolic pathway and might interact with neuromelanin within these organelles.

Interaction of neuromelanin with iron and other metals

High concentrations of iron and other non-alkaline metals are present in several brain nuclei.[58 59] Neuromelanin from the substantia nigra can interact with many heavy metal ions such as zinc, copper, manganese, chromium, cobalt, mercury, lead, and cadmium; in addition, it binds iron particularly strongly.[4 24 25 60 61] In the course of Parkinson's disease and related syndromes, the concentration of iron in the substantia nigra increases by 30–35%.[62 63] This accumulation of nigral iron seems to occur within the neuromelanin granules: the concentration of iron in these granules is higher in patients with Parkinson's disease than in normal subjects.[64 65]

Although a neuromelanin–glycolipid complex was proposed as a good chelating and insolubilising system to bind iron ions,[56] it now appears that iron is bound to catecholic groups and not to lipids. EPR studies showed that in the substantia nigra the ferric iron is bound to neuromelanin as a high spin complex with an octahedral configuration.[4 24 66] Mössbauer spectroscopy demonstrates that ferric iron is chelated by the neuromelanin polymer and that the iron sites are arranged in a ferritin-like ironoxyhydroxide cluster form.[57 61 67] x Ray absorption fine structure spectroscopy[68] and infrared spectroscopy[29] studies confirmed that iron in neuromelanin was bound by oxygen derived phenolic groups in an octahedral configuration. In substantia nigra tissue, neuromelanin is only about 50% saturated with Fe(III), therefore maintaining an important residual chelating capability, which can protect against iron toxicity.[4 66]

Neuromelanin can sequester redox active iron ions, reducing the formation of free hydroxyl radicals.[69] Thus, in normal subjects, neuromelanin may play a protective role by inactivating the iron ions that induce oxidative stress. The ability of neuromelanin to chelate other redox active metals such as copper, manganese, chromium, and toxic metals including cadmium, mercury, and lead[4 24 25 60] strengthens the hypothesis that neuromelanin may be a high capacity storage trapping system for metal ions and, as such, may prevent neuronal damage.

Neuromelanin during aging and Parkinson's disease

Neuromelanin accumulates normally with age in human substantia nigra neurones.[2 16 17 70] A neuronal pigment has also been observed in the substantia nigra of adult rats and dogs, and its concentration seems to depend upon age. In very old (23 months) rats, but not in younger animals, neuromelanin granules were detected by electron microscopy; similar results were observed in aged dogs.[71] Neuromelanin granules were also detected in catecholaminergic cerebellar cells of monkeys (*Macaca mulatta*

and *Macaca nemertina*) and their presence correlated with age.[18] In human substantia nigra, the first small, brown neuromelanin granules were clearly discernable at approximately 3 to 5 years of age.[18 72] The neuromelanin content of neurones is highest in individuals in their 60s, after which it decreases[16]; this phenomenon may reflect the neuronal loss observed in these anatomical structures during aging. However, there is no significant loss of catecholaminergic neurones in the substantia nigra of normal subjects until very old ages.[73–75] A new spectrophotometric method indicates that neuromelanin is not detectable during the 1st year of life, but starts to accumulate thereafter, with a continuous linear trend, and reaches a concentration of 2.3–3.7 mg/g of substantia nigra pars compacta in 50–90 year old individuals. Male and female subjects showed the same age trend of neuromelanin concentration. In patients with Parkinson's disease, neuromelanin values were 1.2–1.5 mg/g of substantia nigra pars compacta, which is less than 50% of that seen in age matched controls (L Zecca, 2000, unpublished results). The absolute number of pigmented neurones in the substantia nigra of normal subjects may be dependent upon ethnicity—an Indian population was found to have fewer pigmented neurones than an age matched Western population.[74]

These observations suggest that neurodegenerative disorders characterised by nigral neurone loss, best typified by Parkinson's disease and other parkinsonian syndromes, are not the result of early aging, as hypothesised in the past. Because the neuromelanin concentration in substantia nigra neurones increases, and the number of pigmented neurones appears to be constant over the life span, it seems that neuromelanin accumulates only in a subpopulation of nigral neurones, whereas other dopaminergic neurones remain non-pigmented. The observed decrease in the neuromelanin concentration occurring in the substantia nigra of patients with Parkinson's disease (L Zecca, 2000, personal communication) confirms the loss of pigmented neurones occurring in the substantia nigra of these patients, as has been reported in neuropathological studies.[73 76 77] Other studies indicate that neuromelanin values decrease in the surviving neurones of the substantia nigra during Parkinson's disease.[19 78] This could be the result of reduced neuromelanin synthesis, neuromelanin degradation, or higher vulnerability of the pigmented neurones.

Neuropathological investigations have examined the presence of extraneuronal neuromelanin in subjects with idiopathic Parkinson's disease and MPTP intoxication.[79 80] Most of this extraneuronal neuromelanin is phagocytosed by microglia and is associated with astrocytic and microglial activation. It may be that neuromelanin could be the effector of a chronic inflammation process in the substantia nigra. Although in idiopathic Parkinson's disease the neurones are depleted in both the substantia nigra and locus coeruleus, in MPTP intoxicated subjects, locus coeruleus neurones are

spared.[80] Such a different neuronal vulnerability might eventually be explained by structural differences in the neuromelanin of the substantia nigra and locus coeruleus.

Although neuromelanin may play a cytoprotective role by sequestering redox active metals, toxic metals, and organic toxic compounds,[81] neuromelanin might also become a source of free radicals by reaction with hydrogen peroxide.[60] When free neuronal iron increases to the point where neuromelanin becomes saturated and it starts to catalyse the production of free radicals, neuromelanin would become cytotoxic.[69] Moreover, because hydrogen peroxide can degrade neuromelanin, the pigmented neurones could loose this putatively protective agent. The consequence may be a release of iron and other cytotoxic metals or compounds from neuromelanin that could accelerate neuronal death.[66]

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