Discussion

Neuromelanin: Past, Present, Future

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The "International Colloquium on Neuromelanin and Parkinson's Disease", held in Sorrento (Naples, Italy) on the 6th - 8th May of this year, (Chaired by Prof. G. Prota) gave me the occasion to make the state of art about neuromelanin structure, function and involvement in neurodegenerative syndromes such as Parkinson's disease. As we will discuss later in this review we expect much from future work concerning neuromelanin function and structure, in fact past and present work reports have not yet provided conclusive results about those topics.

Some more progress has been achieved as concerns the involvement of neuromelanin in drug, for instance MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) or metal caused parkinsonism in primates and man (1-6).

PAST:

Since the first description of a dark brown pigment occurrence within the brain stem of humans by d'Azur (7), much time has flown before an experimental approach to investigative the biochemical nature of the brain pigment was performed. Especially the pigment found in the substantia nigra and in the locus coeruleus catecholaminergic neurons rised much scientific interest, due to the loss of pigmented neurons in some neurodegenerative diseases (8-9). A recent review on the different aspects of neuromelanogenesis may be found in ref. 7.

The brain melanin chemical composition was investigated by several authors and was found to differ considerably from the other known melanins (10-15); moreover neuromelanin was reported to be the oxidation product of dopamine and norepinephrine, however also L-Dopa (L-3,4-dihydroxyphenylalanine), epinephrine and serotonin can be incorporated into neuromelanin (11-17), that when bleached, is similar to lipofuscin (18). No definitive conclusions have been obtained from spectrometric, biochemical and histochemical studies on neuromelanin, neither as concerns the chemical composition nor as regards the synthesis, enzymatic or by autooxidation from catecholic and indolic precursors (17).

The phylogenetic distribution of neuromelanin in Mammalia has been reviewed and investigated within 49 species from 11 orders (19), however due to the technique used it has been questioned that all of those species investigated have neuromelanin (20). In Primates this pigment is well evident and they share with man similar neuromelanin related disorders (21).

The occurrence of pigmented neurons has been reported in Amphibians too, where they may have roles similar to those they have within man and Primates (22).

The amount of neuromelanin increases during ontogenesis, even if there is a wide range of reported appearances, from midterm gestation to 4 years of age in the substantia nigra, while in the locus coeruleus from 5 months of gestation to 3 years of age. At about 30-40 weeks of gestation neuromelanin becomes evident in the dorsal nucleus of vagus (20). During the life neuromelanin increases in the dopaminergic neurons up to 60-70 years and after, the cellular content decreases (23); the changes of cellular neuromelanin content varies linearly with age up to about 70 years (23). The accumulation of neuromelanin with ageing would be responsible for the pigmented neurons degeneration in older people and perhaps in some individuals (Parkinson's disease affected); the accumulation of this substance may result in the decrease of ribosomal RNA content and finally into

nucleolar lesions (23). Other authors think that catecholamine metabolism per se, due to quinones, radicals ecc., i.e. cytotoxic species, may be detrimental to the dopaminergic cells (24).

Neuromelanin is compartmentalized in granules showing tripartite and globular structure consisting of vesicular bodies protruding from a granular or linear lattice (18). A lower amount of pigment, with respect to normal individual, has been found the substantia nigra of phenyl-pyruvic oligophrenics (25).

Many researches spent energies in looking for an enzyme able and responsible for neuromelanin synthesis, however the following findings supported a conflicting enzymatic origin of the pigment: a) albinos show pigmented substantia nigra (26): b) no clear neuromelanin production by substantia nigra or locus coeruleus homogenates was observed (17); c) some brain tissues, other than pigmented nuclei, promote incorporation of melanin precursors (17); d) histochemical evidence as been provided of the occurrence of tyrosinase-like activity in the substantia nigra (27); e) electrophoretic evidence has been reported of the occurrence of a tyrosinase - like activity, phenylthiourea sensitive, able to oxidize L-DOPA, dopamine and 5,6-dihydroxyindole, in human substantia nigra, that is lower in the youngest and Parkinson's disease affected individuals, if compared to the normal mid-age ones (28). The enzyme involved are monoamine oxidase, peroxidase and tyrosinase (17,26-27); however no conclusion was obtained until now.

Many putative roles have been assigned to neuromelanin, as ion metal scavenger (29), phonon-electron coupler (30), drug scavenger and releaser (6), free radical sinker (31), biocybernetic function (32), however other hypotheses have been provided, i.e. the activation of tyrosine-hydroxylase (EC 1.14.16.2) by melanin as a polyanion (33) similarly to the polyanion activation of phenylalanine hydroxylase (34). In this respect it is interesting that tyrosine hydroxylase activity is greatly decreased in the nigro-striatum of Parkinson's disease affected people (33,35-36).

PRESENT:

In the recent few years some new insights concerning neuromelanin chemical structure have come from some european and japanese laboratories (13-15), but as we will discuss later, even if novel components of neuromelanin have been found, such as cysteinyl-dopa, 5-S-cysteinyl-dopamine or an indole derivative of cysteinyl-dopamine or cysteinyl-dopa, bound to palmitic acid (14), the question is not yet settled. The japanese group failed to find incorporation of cysteinyl-dopamine into neuromelanin and its presence in human substantia nigra melanin (13), although the occurrence of cysteinyl catechols derivatives in neuromelanin may explain the pheomelanic component of this pigment. The lack of cysteinyl-dopamine incorporation in neuromelanin rises very intriguing questions.

The apparent present state of the art, as concerns neuromelanin chemical structure, is the same of the past one, uncertainty: does human substantia nigra neuromelanin contain sulfur aminoacid deriving moieties or not? The findings reported above were mainly obtained from degradative studies, followed by HPLC, pyrolysis-GC, and MS. None, at the present, has answered the points whether neuromelanin is auto-oxidatively produced or synthesized under enzymic control, as expected for substances with possible important functions (28).

The present neuromelanin age appears to be more fruitful of studies about the implication of radical species, such as active oxygen, electrophilic species scavengers such as GSH or cysteine and about the enzymes involved in these scavenging processes such as SOD (superoxide dismutase), catalase, peroxidases, glutathione-S-transferase, glutathione reductase ecc. due to the hypothesis that Parkinson's disease and related conditions may be the result of oxidative stress (38). This point have been largely discussed at the Sorrento meeting on "Neuromelanin and Parkinson's disease" by many speakers, so that, one of the main conclusion, that might be drawn from that conference, was the detrimental function of neuromelanin. This point will be the object of some consideration in the next section of this review.

Only a few reports exist on the null role of neuromelanin in Parkinson's disease and among them, the interesting involvement of bFGF-like proteins (basic fibroblast growth factor) in the substantia nigra neuron degeneration proposed by McGeer et al. (39).

Most of the present concern in neuromelanin, free radicals and related neurodegenerative diseases sprang from the MPTP induced parkinsonism (2), the cytotoxicity of catecholamine oxidation products (24), and the free radical content of melanins (40-42); however many agents such as drugs, metals, viruses, CO and trauma, may produce parkinsonian symptoms, thus we expect much from the future research findings to clear both the genesis, the chemical and spacial structures, the normal and pathological functions of neuromelanin.

THE FUTURE:

As we have previously discussed, virtually large room exists for neuromelanin investigations. No conclusive findings have been obtained as regards the neuromelanin molecular structure, the compartmentalization of neuromelanin synthesis, and as whether or not, the derangement of intracellular neuromelanin compartmentalization may be responsible for cytotoxic species efflux, from the neuromelanogenesis sites to the rest of the cell, resulting this in enzyme, genome or structural damages, that may cause a pigmented dopaminergic neuron loss; however this is only a face of the medal involving neuromelanin in Parkinson's disease. Recent reports suggest that neuronal loss in the substantia nigra may be related to the ageing process, affecting the dopamine transporter mRNA levels after the fifth decade, when a steep decrease of tyrosine hydroxylase mRNA, that declines linearly with age, is found (43). An important finding moreover comes from studies on the tyrosine hydroxylase, substance P and calbindin D28K distribution within cell tiers in the substantia nigra, where the most vulnerable cells in Parkinson's disease appear to be the less rich of neuromelanin (44), in spite of reports that suggest a higher vulnerability of the most pigmented neurons in the substantia nigra (45).

The findings reported above rise the questions whether or not neuromelanin has some role in the loss of dopaminergic neurons; in fact the idiopathic abnormal decrease of dopamine transporter and/or tyrosine hydroxilase in the substantia nigra pigmented cells does not seem to be correlated to the neuromelanin content of neurons, in fact the most vulnerable ones seem to be the less rich of pigment. As we can see no clear relation appears between neuromelanin and dopaminergic neuron death. If we give confidence to Nagatsu et al. (33), we should expect that neuromelanin is not detrimental but useful due to tyrosine hydroxylase activation. Mc Geer pointed out, at the Sorrento Colloquium, that great research efforts are displayed to demonstrate the negative function of neuromelanin while, very few people claims that neuromelanin should have a positive function in the pigmented neurons physiology. A major question is: why has not neuromelanin been discarded by natural selection? Why does neuromelanin occur within the highest phylogenetic levels (even if pigmented neurons occur within Amphibia but not easily traceable back to substantia nigra neurons)? Which is the role of bFGF-like proteins in the physiopathology of the nigro-striatal pathways? Does exist any evidence that acatalasia (Takahara disease), progeria, peroxidase or SOD deficiencies are linked with a higher incidence of Parkinson's disease and parkinsonisms? Is neuromelanin synthesized under enzymatic control? We have recently found a melanogenic protein fraction from the rabbit and mouse brain and also shown that purified dopamine-ß-hydroxylase (EC. 1.14.17.1.) has some catecholamine oxidase activity (46).

To my opinion the future research in the neuromelanin field should first discover the physiological function of this substance(s), if any, and subsequently demonstrate that it is a detrimental phylogenetic result; cells are not detrimental to the organism but cancer indeed.

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