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INVITED NEL REVIEW

# Diffuse neuroendocrine system and mitochondrial diseases: molecular and cellular bases of pathogenesis, new approaches to diagnosis and therapy

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**Abstract** Structural and functional alterations of mitochondria have been shown to be responsible for a wide variety of clinical disorders that are referred to as "mitochondrial diseases." It is now obvious that many factors are involved in transport of mitochondrial proteins including cytokines, chaperones, chemokines, neurosteroids, ubiquitin and many others. At the same time the participation and the role of biogenic amines and peptide hormones (which are produced by the diffuse neuroendocrine system cells located in different organs) in endogenous mechanisms of mitochondrial diseases are still unknown. Taking into account the wide spectrum of biological effects of biogenic amines and peptide hormones, and especially their regulatory role for intercellular communication, it seems important to analyze the possible participation of these molecules in the pathogenesis of mitochondrial disorders as well as to draw up new ways for elaboration of new markers for lifetime diagnosis, definition of prognosis and efficiency of specific therapy in neurodegenerative diseases.

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## 1. Introduction: mitochondria are key beachheads of cell pathology

Mitochondria are the sites of crucial cellular functions in eukariotic cells responsible for converting energy derived from chemical fuels by harnessing this energy for biological purposes through a chemiosmotic coupling. Moreover, mitochondria have also been suggested as an important source of cellular second-messenger molecules (reactive oxygen intermediates and others), which are involved in many gene regulatory pathways. In metabolically active cells, mitochondria are the most abundant organelles, and up to 10-20% of the total intracellular proteins have been estimated to be present within this organelle [1].

In spite of mitochondria having their own DNA encoding mt tRNA, mt rRNA and several polypeptides, they import virtually all of their proteins from the cytoplasm. This import process faces the challenge to route the proteins to their correct submitochondrial compartment, and this process requires that most of them must be transported across two membranes. This challenge is met by the joint action of two distinct protein transport systems, one in the outer membrane and the other in the inner membrane.

Mitochondrial precursors are recognized by specific receptors on the mitochondrial surface, which deliver the bound precursors to a protein import channel in the outer membrane. The receptor and channel each consist of several non-identical subunits referred to as ***T**ranslocase **O**uter **M**embrane* (Tom) subunits [2]. One of these components, Tom20, is a major receptor of the mitochondrial translocation complex in *Saccharomyces cerevisae* and *Neurospora crasse* which plays a key role in the biogenesis of mitochondria as a subunit of the translocase of the outer mitochondrial membrane which directly recognizes most cytosolic mitochondrial protein precursors [3]. Although little is known concerning the components of this mitochondrial machinery in mammals, recently the structure of the human gene encoding the homolog of the mitochondrial outer membrane receptor Tom20 has been described [4, 5].

Primary defects in mitochondrial function are involved in over 100 diseases, and the list continues to grow. Structural and functional alterations of mitochondria have been shown to be responsible for a wide variety of clinical disorders that are referred to as "*mitochondrial diseases*" or "*mitochondrial cytopathies*" [6, 7]. It has become apparent that genetic defects in the synthesis of mitochondrial proteins may be the underlying cause of diseases affecting organ systems including the nervous system, skeletal and cardiac muscle, liver and others. These genetic defects can be classified into two major groups; defects of mitochondrial DNA and defects of nuclear DNA. Furthermore, defects of nuclear DNA can cause mutations in genes encoding enzymes or mutations affecting translocase subunits of mitochondrial proteins and defects in intergenomic communication [8]. Many defects of mitochondrial DNA have been reported, but there is a group of inborn mitochondrial myopathies which are associated with deficiencies of many mitochondrial enzymes, hinting at a primary defect of the mitochondrial import machinery.

It is now obvious that many factors are involved in transport of mitochondrial proteins including

cytokines, chaperones, chemokines, neurosteroids, ubiquitin and many others [9–11]. At the same time the participation and the role of biogenic amines and peptide hormones (highly active biologically substances, which are produced by neuroendocrine cells located in different organs) in endogenous mechanisms of mitochondrial diseases are still unknown. Taking into account the wide spectrum of biological effects of biogenic amines and peptide hormones, and especially their regulatory role for intercellular communication, it seems important to analyze the possible participation of these molecules in the pathogenesis of mitochondrial disorders.

## 2. Diffuse neuroendocrine system: evolution of knowledge

In the late 1960s Everson-Pearse suggested that a specialized, highly organized cell system should exist in organisms, whose main feature was the ability of component cells to produce peptide hormones and biogenic amines. The concept was based on an extensive series of experiments for distinguishing endocrine cells in different organs, identifying endocrine cell-generated products and performing a thorough cytochemical and ultrastructural analysis of these cells [12].

A variety of cell types, widely dispersed throughout the organism, have a common ability of absorbing monoamine precursors (5-hydroxytryptophan and L-dihydroxyphenylalanine) and decarboxylating them, thus producing biogenic amines. That ability accounts for the term APUD, an abbreviation of "Amine Precursor Uptake and Decarboxylation" used by Pearse to designate the cell system [13].

To date, the APUD system includes over 60 types of cells located in gut, pancreas, urogenital tract, airway epithelium, pineal gland, thyroid gland, adrenals, adenohypophysis and hypothalamus, carotid body, skin, sympathetic ganglia, thymus, placenta and other organs [14]. Meanwhile the advent of radioimmunological methods and the rapid development of immunohistochemistry resulted in the establishment of a completely unexpected phenomenon, i.e., the same biogenic amines and peptide hormones were identified in neurons and endocrine cells [15].

The accumulated data did not fit the traditional concepts of hierarchical dependence within two main regulatory systems, viz., the nervous and endocrine systems. It became more and more evident that the mechanism of biological regulation should be based on the coordinated functional interaction between the endocrine system and the central and peripheral nervous system considering the common type of information perception and transmission at subcellular, cellular and tissue levels. Recent data on identification of the same and similar physiologically active substances, acting within the nervous system as neurotransmitters and neurohormones, and locally or remotely as hormones within the endocrine system, enables both system to be incorporated into the universal *diffuse neuroendocrine system* (DNES) [14–17]. Actually, it should be possible to unite in the organisms the structurally isolated nervous and endocrine systems by means of functional relationships between biogenic amines and regulatory peptides and, to a certain extent, to provide a basis for the concept of integrated functions. Located in practically all organs and producing biologically active substances, the DNES cells are regulators of homeostasis acting via neurocrine, endocrine and paracrine mechanisms [18, 19].

It is well-known that the nervous and endocrine systems have well-established and very closed related interrelations to regulate systemic homeostasis that involves the production and secretion of a variety of cellular mediators known as *regulatory peptides* (peptide hormones, cytokines, chemokines, integrins and others). Peptide hormones, cytokines and other related molecules regulate homeostasis in the tissue of origin, either via local actions or by recruitment of external systems that facilitate restoration of local homeostasis.

The studies on isolated-cell systems have confirmed that many regulatory peptides and biogenic amines are expressed within the brain. There are many peptidergic neurons and glial cells in the brain which can produce peptide hormones and biogenic amines; also besides neurons, immune cells, such as macrophages, T-lymphocytes, eosinophilic leukocytes and mast cells, which invade the brain after injury or inflammation, are a rich source of cytokines and other active molecules [20–23].

The aim of this review is to analyze the possible role of regulatory peptides and related molecules in endogenous mechanisms of neurodegenerative processes and to identify avenues for further research.

## 3. Neuropathology of Alzheimer's and Parkinson's diseases

The most important diseases among all mitochondrial disorders are the neurodegenerative diseases, including most notably Alzheimer's disease (AD) and Parkinson's disease (PD). AD is characterized by a progressive loss of memory, resulting in dementia and death. AD affects over 20 million

people worldwide and its incidence is expected to double over the next 30 years [24]. A triad of neuromorphophysiological features characterize AD and include amyloid-β plaques (senile plaques), neurofibrillary tangles and extensive neural loss particularly in the hippocampus and cerebral cortex [25]. These changes are associated with dementia and characteristic neurobehavioral consequences. The signs of the disease differ among individuals with the majority of cases arising sporadically, and commonly they have a late life onset (after 65 years of age). In a less common form of familial AD, the onset of the condition is typically much earlier (40-50 years of age) [24].

Parkinson's disease (PD) is a major neurodegenerative disorder with a prevalence of roughly 150 cases for every 100,000 elderly people. The condition is characterized by the progressive deterioration of the dopamine containing neurons in the pars compata of the substantia nigra in the brain stem; the loss of these catecholaminergic neurons is associated with a variety of sensory and motor impairments which lead to tremor, rigidity and akinesia [26]. For an individual to manifest signs of PD, it is estimated that the nigro-striatal dopaminergic neuronal population must be depleted by at least 80%. Thus, in most cases the initiating factor for PD probably precedes the overt signs of Parkinsonism by 5–10 years [26].

## 4. Pathogenesis of neurodegenerative diseases: related molecules and cellular bases

**4.1. Cytokines.** Major cytokines for brain function are neurotrophins (BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; and GDNF, glial-derived neurotrophic factor) and neuropoietins (especially interleukin-6, IL-6). They participate in the mechanisms of growth and differentiation of neurons and in neurotransmission [27]. The most abundant source of cytokines, particularly after local damage, appears to be activated microglia, although neurons, astroglia, perivascular and endothelial cells can also express cytokines [28].

Studies on the localization and expression of peptide hormones and cytokines in response to specific stimuli have important implications for their actions in the CNS. For example, it is clear now that cytokine expression is upregulated rapidly in situations of tissue stress, and that cytokines have important actions that are consistent with their role in restoration of tissue homeostasis. Cytokines have been reported to influence many central neurotransmitters, including noradrenaline, serotonin, GABA and expression of a number of neuropeptides (somatostatin, substance P, opioids, VIP, etc.) in several brain regions [29]. However, the interrelationships between each of these varied neurotransmitter responses and their relevance to specific cytokine actions have yet to be defined. Similarly, a number of second messenger systems in neurons are affected not only by cytokines, but melatonin and other hormones and mediators, including activation of cAMP, increased activity of protein kinase C, synthesis of nitric oxide, release of arachidonic acid and $Ca^{2+}$ flux [30, 31]. Thus, it now seems likely that the behavior of practically all molecules involved in pathogenesis of AD and other mitochondrial diseases may be under control of regulatory peptides.

Several cytokines have been reported which influence neuronal differentiation and growth as well as acutely modify synaptic plasticity in brain slice preparations. For many cytokines and other peptide molecules conflicting data exist, indicating that many can exert neurotrophic, neuroprotective and neurotoxic actions. As reported, transgenic mice overexpressing IL-6 in astrocytes show marked neurodegeneration, and inhibition of action of IL-1 and IL-6 markedly inhibits the neurodegenerative processes [32]. IL-1 induces expression of β-amyloid precursor protein (β-APP) and adhesion molecules in neural tissue [33].

Many hormones influence cytokine actions: glucocorticoids are potent inhibitors of the synthesis and actions of cytokines; also, melanocyte stimulating hormone and vasopressin attenuate actions of cytokines in the brain, and these peptides, as well as lipocortin, have been implicated in impaired febrile responses to cytokines in aging animals [34].

The pathological presentation of AD, the leading cause of senile dementia, involves regionalized neuronal death and an accumulation of intracellular and extracellular filamentous protein aggregates which form lesions termed neurofibrillary tangles and senile plaques, respectively [25]. Several independent parameters have been suggested as the primary factor responsible for this pathogenesis, including apolipoprotein β genotype, hyperphosphorylation of cytoskeletal proteins, or metabolism of amyloid β.

**4.2. Amyloid β (Aβ).** The view that a relationship exists between amyloid deposits and neurofibrillary lesions remains an important unresolved issue in our understanding of the pathogenesis of AD [35–37]. Amyloid plaques (AP), which are a classical neuropathological characteristic of AD, have been reproduced in transgenic mice. These mice exhibit selective neuronal death in the brain regions that are most affected in AD, suggesting that AP formation is directly involved in AD neuron loss [38]. On the other

hand, non-Aβ component of AD amyloid (NAC) is the second component in the amyloid from brain tissue of patients afflicted with AD [39]. Its precursor protein (NACP) was shown to be a brain-specific protein. NACP was more abundant in the neocortex, hippocampus, olfactory bulb, striatum, thalamus and cerebellum. Confocal laser microscopic analysis revealed that anti-NACP immunostaining was colocalized with synaptophysin-immunoreactive presynaptic terminals, therefore NACP is a synaptic protein, suggesting that synaptic aberration observed in senile plaques might be involved in amyloidogenesis in AD [40].

**4.3. Tau-protein**. There are some data indicating that even small numbers of neurofibrillary lesions are pathological and may represent the early stages of AD. There are also many neurodegenerative diseases with numerous positive filamentous lesions: AD, Parkinson's disease (PD), Down's syndrome, miotonic dystrophy, and others. Tau is a microtubule-associated protein that is involved in microtubule asembly and stabilization [41]. In adult human brain, six isoforms of tau are expressed, which are produced by alternative splicing of mRNA from a single gene located on the long arm of chromosome 17. Tau protein mRNA is expressed predominantly in neurons, with recent reports indicating its additional presence in oligodendrocytes. Within nerve cells tau protein is present mainly in axons [42]. In some recent studies the expression of tau-protein has also been shown in cultured skin fibroblasts from Alzheimer's disease patients [43]. A number of studies have characterized tau filaments in various diseases by electron microscopy and immuno-electron microscopy [44]. Currently, three types of filament morphologies can be distinguished and they have a diagnostic significance (for example, type I more often can be identified in AD, type II more characteristic feature for PD).

Tau pathology is one of the central neuropathological characteristic of a number of neurodegenerative disorders since the events leading to the formation of tau filaments are sufficient to produce nerve cell degeneration [45]. Therefore, an important direction for further study is to find either endogenous and exogenous ways to prevent tau filament formation. In this connection, one of the possible ways to prevent tau filament formation and development of amyloid plaque could be to identify the endogenous mechanisms of interpeptide communications.

It appears that biologically active substances (neuropeptides, cytokines, etc.) have several, probably distinctive, actions on the nervous system: as communicators to the brain of systemic injury and other disorders; as modulators of brain responses to peripheral organs; as neuromodulators and neurotransmitters of the CNS control of systemic host defense responses to disease and injury; and as molecules that inhibit or mediate neurodegeneration and repair in the brain. The relevance of peptide molecules and related protein actions to a variety of neurological disorders is now being determined and has opened a potentially fruitful area of research and therapeutic development.

**4.4. Synuclein proteins.** Several mitochondrial neurodegenerative disorders are characterized by intracellular protein accumulations or inclusions, such as the Lewy bodies (LB) in PD. α-synuclein is a proposed component of LB. It was shown immunohistochemically that LB in brains of sporadic PD patients are strikingly synuclein-positive [46]. In addition to synuclein, LB contain ubiquitin, ubiquitin C-terminal hydrolase, and proteasomal subunits, major components of the cellular protein degradation pathway [47, 48]. The following areas of the brain are often involved in this pathological process: the striatum (putamen), substantia nigra, locus coeruleus, inferior olive, pons, and cerebellum.

Synuclein proteins are produced by three genes [49]. They share a structural resemblance to apolipoproteins. α-synuclein is distinguishable from the other synucleins. It uniquely has a histidine at residue 50 (β has a unique histidine at 65). Recent reports of synuclein immunoreactivity in LB suggest the presence of α but not β synuclein [49]. The structure, function and localization of the synucleins might be subject to regulation by signals associated with synaptic activity and neuritic growth.

In general, the distribution of α-synuclein in the brain is similar to the distribution of brain pathology in AD [47]. Additional portions of the synuclein protein are present in amyloid plaques in AD. A significant increase in cytosolic synuclein immunoreactivity in frontal cortical extracts in early AD cases was reported; it seems possible that α-synuclein might potentiate the long-term development of AD [50].

**4.5. Chemokines.** Chemokines and chemokine receptors in the CNS are constitutively expressed at low levels in astrocytes, microglia and neurons of the developing and adult brain and they are induced by inflammatory mediators [51]. Furthermore, chemokines and their receptors are upregulated in various neuropathology including brain tumors and AD [52]. Cell culture studies support a role for chemokines in the differentiation and migration of brain cells. For example, IL-8 enhances the survival of neurons and

the number of microglial and astroglial cells in rat hippocampal cultures, and it influences neuronal growth in the human brain [52]. Chemokines also modulate angiogenesis or neovascularization in lesion brain areas. Moreover, an upregulation of the CXCR2 protein (receptor for IL-8) occurs in senile plaques adjacent to the hippocampus in the brains of AD patients. Because IL-8 promotes survival of hippocampal neurons, a possible involvement of IL-8/CXCR2 in compensatory and reparative mechanisms in the Alzheimer's brain should be considered [51].

**4.6. Integrins.** Integrins are the major family of cell surface receptors that mediate attachment to the extracellular matrix, and specific classes of integrins also mediate important cell-cell adhesive interactions. These integrin-mediated adhesive interactions are intimately involved in the regulation of many cellular functions, including embryonic development, tumor cell growth, programmed cell death, hemostasis and many others [53]. It appears that multiple receptor systems can synergize with integrins to regulate cell proliferation, motility, secretion, and other cellular events. The signalling proteins activated by these synergistic agents are common to many receptor pathways. Thus, although unique pathways may be activated by individual classes of receptors, crosstalk between integrins and other receptor pathways is critically involved in the integration of signals that converge on cells in their natural environments *in vivo*.

**4.7. Chaperones.** Molecular chaperones (Hsp28, α B-crystallin) are also involved in AD [54]. Detailed insights into the role of molecular chaperones have come from studies of mitochondrial protein biogenesis, a process in which chaperones act as unfoldases, pulling devices, and foldases. One of the chaperones is mitochondrial import stimulation factor (MSF) [55]. It seems this factor is a conformational modulator of mitochondrial precursor proteins. Other studies showed that some heat shock proteins (Ssa1p, Ssa2p and especially Hsp70) are involved in the import of proteins into mitochondria, as well as into the endoplasmic reticulum and nuclei [56, 57].

**4.8. Cytochrome c oxidase.** Mounting evidence suggests that defects in energy metabolism contribute to the pathogenesis of AD. Cytochrome c oxidase is kinetically abnormal, and its activity is decreased in brain and peripheral tissue in late-onset AD [58]. Coenzyme Q might be a candidate for this; its level was decreased relative to cytochrome oxidase. As a sign of the aging process, the number of cytochrome c oxidase-negative skeletal and heart muscle fibers increased with age. In addition, evidence has been presented for age-related changes in coenzyme Q levels that are corresponded with apoptosis in several tissues in rats and in humans [59].

**4.9. Neurosteroids.** It is now established that the brain itself also synthesizes steroids *de novo* from cholesterol in a variety of vertebrates [60]. In the brain, glial cells play a major role in neurosteroid formation and metabolism [61]. Purkinje cells produce neurosteroids (pregnenolone and progesterone). These cells demonstrate immunopositive staining with antibody to key steroidogenic enzyme cytochrome P450scc [62]. Progesterone (one of the main neurosteroids) is shown to be produced from pregnenolone by Schwann cells in peripheral nerves, and some observations indicate a role for locally produced progesterone in myelination, demonstrate that progesterone is not simply a sex steroid, and suggest a new therapeutic approach to promote myelin repair [63]. Mitochondria of C6-2B glioma cell line participate in the biosynthesis of pregnenolone converting (22R)-22-hydroxycholesterol to pregnenolone by a mechanism blocked by aminoglutethimide [64].

**4.10. Oxygen radicals.** Many diseases related to aging may involve oxygen radicals at some stage in their development. In these diseases, it has been proposed that mutations of mthDNA and changes in cellular bioenergetics contribute in some way to the aging process and to the development of degenerative diseases [65]. Though only recently uncovered as a physiologic messenger, nitric oxide (NO) is increasingly appreciated as a major regulator in the nervous, immune, and cardiovascular systems [66]. Besides mediating normal functions, NO has been implicated in many different pathophysiologic states including neurodegenerative diseases [67].

Of all the organs in the body, the central nervous system (CNS) takes more than its share of oxidative abuse [68]. The reasons for this are several-fold. The brain although constituting only a small percentage (in the human about 2%) of the body weight consumes a disproportionately large amount (in the human about 20%) of the $O_2$ inhaled. Given that by-products of $O_2$ are toxic, it is not suprising that neural tissue may thus be destroyed at a more rapid rate than other organs.

Mitochondrial DNA (mtDNA) has more than 10 times the level of oxidative DNA damage than does nuclear DNA [67]. This increase may be due to a lack of mtDNA repair enzymes, a lack of histones protecting mtDNA, and the proximity of mtDNA to oxidants generated during oxidative phosphorylation. The cell defends itself against this high rate of damage by a constant turnover of mitochondria, thus presumably removing the damaged mitochondria that produce increased oxidants. Despite this turnover, oxidative lesions appear to accumulate with age in mtDNA at a higher rate than in nuclear

DNA. Oxidative damage could also account for the mutations in mtDNA that accumulate with age [69].

That oxidative stress may be a culprit in neuronal loss in AD has been emphasized in recent years and the evidence is becoming progressively stronger that radicals are involved in the neural pathogenesis of AD [70]. The free radicals that have been incriminated as causing neuronal loss are believed to be generated by Aβ [69].

According to the free radical theory of PD, dopaminergic neurons are lost as a consequence of their relatively high exposure to reactive oxygen species, most notably $H_2O_2$ which is produced during both the enzymatic, via monoamine oxidase activity, and non-enzymatic, due to the auto-oxidation, destruction of dopamine [71]. Not only does oxidative stress destroy the dopaminergic neurons but it also compromises mitochondrial oxidative phosphorylation leading to decreased energy output by these organelles and eventually to secondary death of the cells.

**4.11. Glutamate.** There is an increasing amount of experimental evidence that oxidative stress is a causal, or at least an ancillary, factor in the neuropathology of several adult neurodegenerative disorders [72]. At the same time, excessive or persistent activation of glutamate/gated ion channels may cause neuronal degeneration in these same conditions. Glutamate and related acidic amino acids are thought to be the major excitatory neurotransmitters in the brain and may be utilized by 40 percent of the synapses [73]. Thus, two broad mechanisms, oxidative stress and excessive activation of glutamate receptors, are converging and represent sequential as well as interacting processes that provide a final common pathway for cell vulnerability in the brain. The broad distribution in the brain of processes regulating oxidative stress and mediating glutamatergic neurotransmission may explain the wide range of disorders in which both have been implicated. Yet differential expression of components of the processes in particular neuronal systems may account for selective neurodegeneration in certain disorders. Although NO participates in normal synaptic transmission, excess levels of NO are neurotoxic. NO stimulates glutamate neurotoxicity which may contribute to dysfunction in neurodegenerative diseases such as Alzheimer's and Huntington's diseases.

Evidence is now emerging that activation of glutamate-gated cation channels may be an important source of oxidative stress and that these two mechanisms may act in a sequential as well as a reinforcing manner, leading to selective neuronal degeneration. Understanding the relation between oxidative stress and glutamate neurotransmission could lead to the development of pharmacologic interventions that disrupt this chain of pathological events without impairing excitatory neurotransmission [73].

**4.12. Calcium homeostasis.** Brain aging is associated with a marked decline in mental faculties. One hypothesis postulates that sustained changes in the regulation of intracellular $Ca^{2+}$ concentration are the major cause of neuronal degeneration [74]. This "calcium hypothesis" is supported by demonstration of the impairment in aged neurons of molecular cascades that regulate intracellular $Ca^{2+}$ concentration. The conceptual pillars of this point of view are dysfunction of intracellular $Ca^{2+}$ homeostasis and neuronal loss [75]. This view of the aging brain is that the decrease in cognitive function results mainly from neuronal death and that this leads to a decrease in the number of brain cells. Strong support for this hypothesis has come from studies of neurodegenerative diseases, such as AD. In AD there is a profound loss of neurons that correlates well with the decrease in learning abilities and memory function [25]. In addition, a key element of AD pathology, the accumulation of Aβ, has been shown to disrupt neuronal intracellular $Ca^{2+}$ homeostasis [75]. The brain contains a huge population of glial cells that are responsible for the regulation of the brain microenvironment. They can also play an important role in the integrative function of neurons by controlling the concentrations of neurotransmitters and neuromodulators, and thus affecting synaptic transmission. Glial cells, especially astrocytes, rely heavily on neuronal intracellular $Ca^{2+}$ homeostasis, signalling that is involved in most of their response to neurotransmitters [61].

**4.13. Apoptosis.** In any case, the death of neurons is a final stage of neurodegenerative diseases and this phenomenon is known as apoptosis [76]. During the development of the vertebrate nervous system, up to 50 percent or more neurons normally die soon after they form synaptic connections with their target cells [77]. *Bcl*-2 and related proteins have become a major focus of efforts to unravel the intracellular molecular events that regulate cell survival, and cause cell death [78]. Clarification of the repertoire and functional significance of the interactions between these proteins, and identification of the chain of molecular events in which they fit, will greatly increase our understanding of the apoptotic process. Moreover, neurons are particularly useful for studying the regulation of cell survival and apoptosis because, being postmitotic cells, experimental analysis is not complicated by cell proliferation. Furthermore, the roles of *bcl*-2 related proteins in

certain neurons might have important therapeutic implications for neurodegenerative diseases [79].

The intracellular membrane-bound protein *bcl*-2 is probably associated with the cytoplasmic surface of the nuclear envelope, endoplasmic reticulum, and mitochondria [80]. Experimental over-expression of *bcl*-2 prevents the death of neurons deprived of particular neurotrophic factors *in vitro*, and rescues developing neurons that would otherwise die *in vivo*.

The intracellular localization of *bcl*-2 to the inner mitochondrial membrane [81], endoplasmic reticulum membrane and the nuclear envelope has led to several hypotheses about how it might work. The localization of *bcl*-2 in mitochondria has raised the possibility that it might protect against apoptosis by altering mitochondrial function. The localization of *bcl*-2 to major sites of oxygen free-radical generation, and evidence that reactive oxygen species might be involved in causing apoptosis in neurons [82] and other cells have raised the possibility that *bcl*-2 might prevent apoptosis by either acting as an antioxidant or by inhibiting production of free radicals [83].

## 5. Hormones in the brain: localization and role for central nervous functions

**5.1. Melatonin.** During the last decade a great deal of attention has been focused on melatonin, one of the hormones of the DNES, which for many years was considered exclusively as a secretory product of pineal gland [84, 85]. As soon as highly sensitive antibodies to indolealkylamines became available [86], melatonin was identified not only in the pineal gland, but also in extrapineal tissues, i.e. retina, cerebellum, gut mucosa, airway epithelium, kidney and other tissues [87] as well as in non-neuroendocrine cells such as mast cells, natural killer cells, eosinophilic leukocytes, platelets and endothelial cells [87] and in bone marrow cells [88]. Also it has now been shown that many cells in different organs possess melatonin receptors and a variety of melatonin receptors have been identified in many areas of the human brain [89]. The above list of cells which contain melatonin indicates that this MT indoleamine has a unique position among the hormones, being found in a variety of organ systems including the CNS. Functionally, melatonin-producing cells are likely to be part and parcel of the DNES as a universal system of response, control and organismal protection. Taking into account the large number of melatonin-producing cells, the wide spectrum of biological activities of melatonin and especially its properties as a regulator of biological rhythms and antioxidant, extrapineal melatonin may be an important paracrine molecule to ensure optimal cellular function and protection.

The identification of melatonin in the pineal gland and in extrapineal tissues stimulated interest in the physiology of this hormone and a wide spectrum of biological activities of melatonin has been uncovered. Some of these functions include the control of biological rhythms, seasonal reproductive events, stimulation of immune processes, cytostatic and antiproliferative effects in vitro and in vivo [85].

Additionally, another unexpected function of melatonin was uncovered. Thus, the indole has been shown to be a free radical scavenger and antioxidant [72, 90, 91]. Melatonin is now known to be a potent hydroxyl radical scavenger and under some circumstances it protects against free radical damage more effectively than the well-known scavenger glutathione [92]. Melatonin is now known to scavenge the highly toxic hydroxyl radical, the peroxynitrite anion, singlet oxygen and NO. Also, secondarily, it reportedly scavenges the superoxide anion radical [92]. Additionally, it stimulates mRNA levels for superoxide dismutase and the activities of glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase (all of which are antioxidative enzymes), thereby increasing its antioxidative capacity [93]. Also, melatonin inhibits nitric oxide synthase, a pro-oxidative enzyme and stimulates the rate limiting enzyme in glutathione synthesis, α-glutamyl-cysteine synthase [94]. There is ample evidence that the brain of PD patients exhibits signs of enhanced oxidative stress. Acuna-Castroviejo et al. [95] have investigated the ability of melatonin to protect the brain against the toxic effects of MPTP, a drug that produces Parkinson like signs. In this model system, melatonin was strongly protective. Also, Mayo and co-workers [96] assessed the ability of MT to protect against dopamine autoxidation-induced protein damage using the oxygen radical absorbance capacity assay. The results showed that melatonin reduces the degree of oxidation of the fluorescent protein which is the basis for the assay, indicating that melatonin prevents macromolecular damage that is a result of dopamine autooxidation. The authors surmised that this was due to the free radical scavenging capacity of melatonin and they suggested that the indole may have beneficial effects in reducing oxidative damage in the brain of PD patients.

While oxidative stress may be one feature that links many neurological deficits, it is also obvious that these diseases have extremely complex etiopathologies and it is unlikely that a single agent will totally combat their development. Moreover, there is an urgent need to understand the mechanisms

underlying the degeneration of neurons.

Melatonin as a potential treatment to defer neurodegenerative diseases is of interest for several reasons: the endogenous production of this molecule falls with age coincident with the onset of many of the age-associated neurodegenerative conditions [93]; and melatonin readily crosses the blood-brain barrier and after its exogenous administration it is found in high concentrations in the brain, sometimes exceeding those in the blood manifold [70]; melatonin is a ubiquitously acting free radical scavenger and antioxidant [90] which in models of neurological diseases has proven effective in reducing oxidative damage and preserving neurological function [72].

The importance of the study of melatonin as a promising molecule to understand better the pathogenesis of AD and PD is illustrated by the recent fact that soluble forms of full-length β-amyloid precursor protein (β-APP) of the Aβ-peptide were detected in secretory granules of chromaffin cells [97], where melatonin and dopamine are also synthesized [87]. Moreover, it was shown that stimulation of APP secretion was paralleled by a stimulation of secretion in catecholamines and chromogranin A, indicating that secretion of APP was mediated by chromaffin granules. Because secretion of APP from primary chromaffin cells was time-dependent, we surmise that melatonin may have a direct effect on this process.

**5.2. Serotonin (5-hydroxytriptamine; 5-HT).** 5-HT neuron and neurotransmitter loss in normal aging and neuropsychiatric diseases of late life may contribute to behavioral changes commonly observed in the elderly population [98]. Extensive evidence implicates a deficit in serotoninergic neurotransmission in the development of major depression. The concentrations of 5-HT are reduced by 18% in the frontal cortex and by 21-37% in the hippocampal cortex, hippocampus and striatum in AD [99]. It has been further suggested that the age-related changes in 5-HT neurons may predispose the elderly to depression. There is also increasing evidence that a disturbance in serotoninergic function may play a role in cognitive impairment in AD.

**5.3. Catecholamines (CA).** There are many data showing a significant loss of dopamine (DA) immunopositive neurons in the brain of PD patients [26]. Also a reduction of DA concentrations (18-27% compared with normal level) have been noted in AD patients in the temporal cortex and hippocampus [99].

Immunocytochemical techniques have been used to compare the proportion of neurons expressing CA in the different brain areas of neurologically normal elderly humans to that of age-matched AD patients [100]. The CA cells in the frontal cortex of the AD patients were found to be significantly decreased; the CA are present in both cortical neurons and astrocytes. Reinikainen et al. [99] showed that in AD patients the concentration of noradrenaline was reduced (18-36% compared with normal patients) in the frontal and temporal cortices, and in the putamen.

**5.4. Histamine.** Histamine is known to be a neurotransmitter, but it has not been clearly implicated in major diseases. All histaminergic neurons reside in the posterior hypothalamus and innervate most brain areas, which is compatible with the concept that histamine is involved in general central regulatory mechanisms. A sensitive high-performance liquid chromatographic fluorimetric method was used to measure histamine content in the post mortem brain in AD patients and age-matched controls [101]. At the same time the cellular storage sites and distribution of histaminergic fibers were examined with a specific immunohistochemical method. The histamine content was significantly reduced in the hypothalamus (42% of control value), hippocampus (43%) and temporal cortex (53%) of AD brains. Histamine concentration in other cortical areas, putamen and substantia nigra were not significantly altered. Histamine-containing nerve fibers were found in the hippocampus, parahippocampal gyrus and subiculum of both AD brains and controls. No histamine-containing mast cells were seen in these temporal structures. Histamine in the human temporal lobe is stored in nerve fibers originating from the posterior hypothalamus, and not in mast cells. A reduction in brain histamine levels may contribute to the cognitive decline in AD directly or through the cholinergic system. Thus, development of drugs that penetrate the blood-brain barrier and increase histaminergic activity may be beneficial in AD [101].

**5.5. Somatostatin (ST).** ST was originally isolated from hypothalamic extracts [102]. It has subsequently been shown to be present in neurons and endocrine cells throughout the brain and gut [103]. Numerous central effects of ST have been described, although there appear to be some conflicts in the literature. The consensus appears to be that the main neurobiological effect of ST results in a generalized arousal, with concomitant enhancement of grooming and exploratory activities. Metabolically, it has been shown to inhibit the hyperglycemic response to a variety of stressors [104]. Disturbances in ST synthesis and secretion may play a role in the patho-

genesis of various neurological diseases. Recent data suggest a disturbance of some brain ST neurons in AD. Moreover, some endocrine activities known to be regulated by ST, such as growth hormone, thyroid-stimulating-hormone, somatomedins, as well as insulin and glucose, also seem to be affected in some patients [105]. It is speculated that these changes are due to a global CNS and endocrine ST defect in AD and that the described endocrine imbalances may indirectly be responsible for at least part of the CNS pathology. A deficiency in ST is the most consistently described neurochemical alteration in AD attributable to intrinsic cortical neurons [106]. ST concentrations are depleted in the cerebral cortex in both AD and in the dementia that accompanies PD [107]. ST neurons in both illnesses are markedly dystrophic and may be reduced in number. Li et al. [108] tried to verify if there is a difference in the number of ST neurons in the cortex between normal aging versus AD patients and, secondly, if any of these neurons were dying via apoptosis. In their specimens, immunohistochemistry revealed that there was no difference in the number of ST-containing neurons between the two study groups. Moreover, the bulk of the apoptotic cells that were identified using the sensitive immunocytochemical TUNEL method, none contained ST [108]. It is concluded that while there is apoptotic cell death in normal aging and AD, it does not seem to occur in neurons which contain ST in any significant amount.

A novel role for receptor-associated protein in ST modulation and its implications for AD was shown recently. It is known that receptor-associated protein appears to play an important role in low-density lipoprotein receptor-related protein (LRRP) trafficking. Since ligands for the LRRP have been implicated in AD and normal functioning of this protein is indispensable for CNS development, deficient LRRP expression may result in CNS alterations [109]. In this study, receptor-associated protein-knockout mice were behaviorally tested and nervous system integrity was assessed via in situ hybridization and immunocytochemical/laser confocal microscopy methods. In wild-type mice, the LRRP was found to be highly co-expressed with ST in hippocampal and neocortical inhibitory neurons. LRRP-knockout mice, however, showed a significant decrease in number of ST-expressing neurons in the CA1 region and ST expression within these neurons. The decreased number of ST neurons significantly correlated with cognitive impairment observed in the receptor-associated protein in modulating the functioning of ST-producing neurons. Furthermore, this has implications for AD pathogenesis, in which altered regulation of both ST and the known LRRP ligands is a consistent finding.

**5.6. Endogenous opiates.** Yew et al. [100] obtained only minimal difference in the proportion of cortical neurons expressing leu-enkephalin between normal and AD patients.

**5.7. Hypothalamic and pituitary peptides.** The neuropathological hallmarks of neurodegenerative diseases are very prominent in the hippocampus [110], a brain site that is pivotal for regulation of the synthesis of the hypothalamic and pituitary hormones. An alteration of neuroendocrine processes is supported by a significantly reduction of adrenocorticotropin hormone (ACTH) levels in cerebrospinal fluid in AD patients as compared with the controls [111]. Several studies indicate a reduction in corticotropin-releasing hormone (CRH) immunoreactivity in the cerebral cortex of AD patients [112, 113], particularly in temporal, frontal, and occipital areas. Nevertheless, these findings are not specific to AD. In fact, reduced levels of CRH in cerebrospinal fluid were also demonstrated in patients with vascular dementia [114], and reduced CRH immunoreactivity in cerebral cortex was found in PD.

An attenuated growth hormone-releasing hormone (GHRH)–induced growth hormone response specific to AD has been demonstrated [115]. Furthermore, a reduction in cerebrospinal fluid levels of antidiuretic hormone was observed not only in AD patients, but also in patients with frontal lobe dementia [116]. No alteration in the synthesis of thyrotropin-releasing hormone and prolactin was found in AD or PD [117].

**5.8. Substance P (SP).** SP was first isolated and chemically characterized from the hypothalamus. Immunohistochemical findings indicate that many nerve fibers from the amygdalo-fugal pathway, probably via the stria terminalis, contain SP, and enter the bed nucleus of the stria terminalis [118] and lateral hypothalamus [119]. Besides, the neurons with SP immunoreactivity have been observed in the arcuate nucleus, ventral and dorsal premammillary nucleus, dorsomedial nucleus, in the medial preoptic area, the periventricular nuclei of the dorsal tuberal region, and the lateral hypothalamus [120, 121]. These cells lack a projection to the median eminence but probably subserve important roles in integrating information from within the limbic system, including neuroendocrine regulation [122]. An important ultrastructural observation is that terminals containing SP form axodendritic synapses in the tuberoinfundibular region [123].

SP immunoreactivity is present also in anterior pituitary and in median eminence [118, 123]. It

seems to be possible that SP plays a role as a paracrine regulator of intrabrain hormonal status [122]. Immunocytochemical studies [100] have not documented a difference between the number and/or functional activity of cortical SP-immunoreactive neurons in healthy and AD patients in the same age.

**5.9. Neurotensin (NT).** Like SP, NT was also first isolated from the hypothalamus. Numerous cells containing NT have been found in the paraventricular and periventricular cell groups and in the lateral hypothalamus. Both magno- and parvocellular neurons are labeled with NT in the paraventricular nucleus, which may indicate that NT-stained cells are components of the hypothalamic-anterior pituitary axis and the neurohypophyseal tract [124]. Scattered NT-positive cells have been observed in other hypothalamic regions, with the exception of the supraoptic, suprachiasmatic, and ventromedial nuclei [125]. Dense fiber labeling is located in the paraventricular and periventricular zones and, importantly, in the median eminence [126].

Some hypothalamic neurons that are positive for NT may also contain CA. These cells are distributed in the periventricular and arcuate regions [127]. Like SP, NT immunoreactivity has been identified in anterior pituitary cells [128]. The role of NT in central neural functions is not defined; one idea is that this peptide together with SP may regulate the content of other peptides in the brain [122].

**5.10. Cholecystokinin (CCK).** The presence of cholecystokinin (CCK) in high concentrations in a number of brain areas, its colocalization with DA in some central neurons, the distinct behavioral effects it has, and the alterations in certain neurotransmitter systems that are seen following its peripheral or central administration, all implicate CCK as a neuromodulator or neurotransmitter [103]. High concentrations of CCK in the CNS occur in the cortex, caudate nucleus and olfactory bulb [129]. The effects of CCK in the CNS may involve its interaction with major neurotransmitter pathways. CCK injection into the lateral hypothalamus increases DA and noradrenaline bindings in the nucleus accumbens [130]. There are some data indicating a decrease in the number of CCK immuno-positive neurons in the cortex of post mortem AD brain [131].

**5.11. Bombesin (BOM).** BOM immunoreactivity is localized in nerve cells in different areas of the brain, but the largest amount of this peptide is present in the hypothalamus and brain tissue closely to fourth ventricle [103]. Like many other gut peptides (e.g. CCK), found in brain, BOM has been shown to reduce meal size. Additionally, it has important neurobiological effects. Brown [132] reported that BOM activates the adrenal medulla and results in markedly elevated plasma adrenaline levels, with secondary increases in plasma glucose and glucagon. Because noradrenaline is reduced in AD [99], it is possible that BOM may have therapeutical significance in maintaining normal levels of noradrenaline.

**5.12. Neuropeptide Y (NPY).** It is known that NPY is found in high concentrations in the cerebral cortex and is contained in cortical neurons [103]. NPY-containing nerve fibers also innervate small blood vessels. NPY is colocalized with catecholamines in some areas of the brain [133], and chemical depletion of catecholamines results in depletion of NPY in some, but not all, neurons [134]. Beal et al. [135] measured concentrations of this peptide in postmortem tissue from AD patients and controls using a sensitive and specific radioimmunoassay. High-performance liquid chromatography showed that more than 95% of immunoreactivity co-migrated with synthetic standards in both AD and control frontal cortex. Significant reductions in neuropeptide Y immunoreactivity were found in the cortex, the hippocampus, and the locus ceruleus. The regions particularly affected included the temporal lobe, frontal lobe, and occipital cortex.

A reduction in immune function has been found in patients with a major depressive disorder and in persons undergoing severe life stress [27]. Irwin et al. [136] investigated the association between NPY and natural killer (NK) cytotoxicity in AD depression. Circulating concentrations of NPY in plasma were inversely correlated with NK activity in AD patients. These findings suggest that the release of NPY may be associated with the modulation of NK cytotoxicity.

**5.14. Insulin (INS).** Earlier it was impossible to imagine the active production of insulin outside of the pancreas, especially in CNS, but in the last few years new evidence has indicated that insulin and its receptors are present in the brain. Insulin was detected in the brain by radioimmunoassay, biochemical and immunochemical methods [137]. Immunohistochemical localization of insulin-containing neurons has been shown in many areas of the brain, but the olfactory bulbs and hypothalamus consistently have the highest concentrations of insulin. Insulin provides for glucose utilization in brain tissue as well as is an important regulatory peptide in the CNS which participates in many physiological processes (e.g. insulin inhibits firing of neurons in the hippocampus and hypothalamus). Several lines of evidence indicate that insulin may influence synaptic activity. Insulin modulates mono-

amine uptake in cultured neuronal cells [138] and stimulates synaptosomal uptake of neurotransmitter amino acids [139].

Insulin increases catecholamine turnover and release from brain cells and has also been shown to stimulate Na, K-ATPase activity in the hippocampus. Acquired disturbances of several aspects of cellular metabolism appear pathologically important in sporadic AD. Among these brain glucose utilization is reduced in the early stages of the disease and the regulatory enzymes important for glucose metabolism are reduced [140]. In the brain, INS, insulin-like growth factors and their receptors regulate glucose metabolism and promote neuronal growth. INS and c-peptide concentration in the brain are decreased with aging and AD [140]. Weak INS-immunoreactivity could be demonstrated histochemically in pyramidal neurons of controls, whereas in AD a stronger INS-immunoreactivity was found. Brain INS receptor densities in AD were decreased compared to middle-aged controls, but increased in comparison to age-matched controls. INS growth factor-I receptor densities were unchanged in aging and in AD. Tyrosine kinase activity, a signal transduction mechanism common to both receptor systems, was reduced in AD in comparison to middle-aged and age-matched control groups [100, 140]. These data are consistent with a neurotrophic role of INS in the human brain and a disturbance of INS signal transduction in the AD brain and favor the hypothesis that INS dependent functions may be of pathogenetic relevance in sporadic AD.

**5.15. Glucagon (GLU).** In addition to being found in the pancreas and gut, GLU immunoreactivity has been demonstrated in the brain, where the highest concentrations appear to be in the hypothalamus with intermediate amounts in the midbrain and low amounts in the cortex [103]. There are not many research data to establish a possible function of GLU in the CNS. GLU immunoreactivity is released from a synaptosomal preparation of the thalamus, hypothalamus, and brain stem in response to $K^+$ [141]. These data support a potential role of CLU as neurotransmitter or neuromodulator, which could be involved in the cascade of molecular reactions that regulate intracellular $Ca^{2+}$ concentration. Many data testify to dysfunction of intracellular $Ca^{2+}$ homeostasis to result in neuronal loss [74, 75].

**5.16. Endothelins (ET).** ET are potent vasoactive peptides produced by endothelial cells that elecit prolonged constriction in most smooth muscle preparations and dilation in others [142]. Of three isopeptides, ET-1 is the only form constitutively released and may modulate vascular tone via binding to one of several receptor subtypes in smooth muscle. ET-1 immunoreactivity in the AD brain was significantly increased in frontal and occipital cortex than those in the control brain and significant correlation was found between frontal and temporal lobe of AD brains [143]. These findings may explain the clinico-radiological results that the cerebral blood flow is decreased in AD patients, the mechanism of which is still unknown.

**Chromogranin A (CGA).** CGA belongs to a multifunctional peptide family widely distributed in secretory vesicles in neurons and neuroendocrine cells. Within the brain, CGA is localized in neurodegenerative areas associated with reactive microglia. CGA stimulated microglial cells to secrete heat-stable diffusible neurotoxic agents and also induced a marked accumulation of NO and tumor necrosis factor by microglia [144]. It seems to be possible that CGA represents an endogenous factor that triggers the microglial activity responsible for the pathogenesis of neuronal degeneration.

## 6. Conclusion: further investigations of brain hormones for improvement of diagnosis and therapy of neurodegenerative diseases.

In spite of many reports on the study of the behavior and role of different biologically active substances in the pathogenesis of mitochondrial diseases, most of them are devoted to concret one or two types of molecules. Thus, complex studies, in which the behavior of many molecules were studied at the same time and at the same patients are absent.

We assume, that it is now essential to identify not only those cytokines and other molecules above and their actions that are associated directly with physiological regulation and disease processes, but also their mechanisms of communications and joint actions. A clear understanding of these processes, combined with development of methods to manipulate them, is likely to offer significant therapeutic potential in the successful treatment of mitochondrial diseases.

It is necessary to underline, that while the patient is alive, the distinction between different forms of dementia rest with clinical assessment. The diagnosis of a specific form of dementia as AD manifestation is confirmed only at autopsy. This circumstance dictates the necessity of the search for lifetime markers.

It seems to be possible because recently the results of some investigations [144, 145] suggest that AD might be a disease not only of the central nervous system, but might be a systemic disorder. If so, the use of human peripheral cells and tissue

biopsies could provide a promising tool for lifetime diagnosis of AD and other neurodegenerative diseases.

We suppose that the further investigations in this direction should include the following steps to better understand the inter- and intracellular mechanisms of neurodegenerative pathology as well as for elaboration of a new promising lifetime marker of these diseases. The main steps follow:

• ***to study the molecular bases of AD and PD associated with defects of the mitochondrial machinery of protein translocation*** (i.e. to complete the sequence of the hTom20 gene; to elucidate the chromosome location of the gene and processed pseudogenes; to identify polymorphism of the hTom20 gene in the general population as well as in patients with AD and PD; to identify new subunits of the translocase of the mitochondrial outer membrane (Tom complex); and to characterize their respective genes).

• ***immunocytochemical mapping and image analysis of microscopic manifestations of AD and PD*** (to identify localization of the most key molecules which are involved in pathogenesis of neurodegenerative diseases in the human brain; to study in detail a functional morphology of cells and tissue structures immunocytochemically positive to molecules above and to compare them to the pathological lesions on light and electron-microscopical levels in postmortem brain in AD and PD patients).

• ***development of new methods for lifetime diagnosis and treatment of AD and PD*** (taking into account an important role of MT as scavenger of free radicals, it seems to be very promising to clarify the role of MT in intracellular mechanisms of neurodegenerative diseases and to elaborate it as a new lifetime marker for diagnostics of AD and PD. To achieve this aim the objectives below should be solved: 1) to identify the key molecules of these disorders (i.e. β-APP, tau-protein, ubiquitin, dopamine, NO-synthase) and MT in human blood lymphocytes from healthy volunteers and patients with AD and PD, and thus to show that blood lymphocytes could be used as a suitable object for lifetime diagnosis of AD and PD; 2) to carry out the quantitative immunocytochemical analysis of concentration of β-APP, tau-protein, ubiquitin, dopamine, NO-synthase and MT in the lymphocytes from AD and PD patients; 3) to determine the excretion in urine of the products of MT interactions with free radicals: cyclic 3-hydroxymelatonin (3-HMT), $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine (AMFK), $N^1$-acetyl-5-methoxykynuramine (AMK) [146] as well as 6-sulfatoxymelatonin (aMT6s) in AD and PD patients; 4) to identify the polymorphisms and mutations in Tom20 gene in human blood lymphocytes of the general population and patients with AD and PD; 5) to study correlations between expression of Tom20 gene, β-APP, tau-protein, ubiquitin, dopamine, NO-synthase and MT in human lymphocytes of healthy people as well as of AD and PD patients and compare them with excretion indices of the same groups; and 6) on the basis of data of the research above to clarify the biological role of MT in intracellular mechanisms of neurodegenerative diseases and to elaborate aMT6s as a new non-invasive marker for lifetime diagnosis, definition of prognosis and efficiency of specific therapy in individual AD and PD patients).

Thus, there is no doubt that DNES and its hormones are multifunctional biologically active molecules, located everywhere in the organism, including the brain play an important role in the pathogenesis of AD, PD, and other neurodegenerative and mitochondrial diseases. The further investigations in this field of study seem to be very effective as well as for elucidation of molecular and cellular bases of pathological mechanisms of neuronal degeneration and moreover for elaboration of optimal methods of diagnosis and therapy of many diseases.

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