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Critical Review

Brain Mitochondrial Dysfunction in Aging

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Summary

Aging of mammalian brain is associated with a continuous decrease of the capacity to produce ATP by oxidative phosphorylation. The impairment of mitochondrial function is mainly due to diminished electron transfer by complexes I and IV, whereas inner membrane H^+ impermeability and F_1-ATP synthase activity are only slightly affected. Dysfunctional mitochondria in aged rodents show decreased rates of respiration and of electron transfer, decreased membrane potential, increased content of the oxidation products of phospholipids and proteins, and increased size and fragility. In aging mice, the activities of brain mitochondrial enzymes (complexes I and IV and mtNOS) are linearly correlated with neurological performance (tightrope and T-maze tests) and with median life span and negatively correlated with the mitochondrial content of lipid and protein oxidation products. Conditions that increased mice median life span, such as moderate exercise, vitamin E supplementation, caloric restriction, and high spontaneous neurological activity; also improved neurological performance and mitochondrial function in aged brain. The diffusion of mitochondrial NO and H_2O_2 to the cytosol is decreased in the aged brain and may be a factor for reduced mitochondrial biogenesis.
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Keywords mitochondrial dysfunction; oxidative damage; life span; complexes I and IV; mtNOS; mitochondrial biogenesis.

Abbreviations complex I, NADH-ubiquinone reductase; complex IV, cytochrome oxidase, mtNOS; mitochondrial nitric oxide synthase; TBARS, thiobarbituric reactive substances.

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AGING AND MITOCHONDRIAL ENERGY TRANSDUCTION

Mitochondria were brought to attention in aging biology due to the central role of mitochondria in producing chemical energy (ATP) to meet cellular requirements and to the declines of basal metabolic rate and of physiological performances which are characteristic of aged mammals. Aging is accompanied by a general decline of physiological functions with those that depend on the central nervous system being more affected (*1*). The higher quality of the integrated responses of the central nervous system has been associated with increased life spans and with decreased neurodegeneration (*1, 2*).

Mitochondrial oxidative phosphorylation is a process that encompasses electron transfer between the complexes of the respiratory chain, vectorial H^+ release into the intermembrane space, and H^+ re-entry to the matrix through F_0 with ATP synthesis by F_1-ATP synthase. An age-dependent impairment of mitochondrial function may be due to: (a) decreased electron transfer; (b) increased H^+ permeability of the inner membrane; and (c) decreased H^+-driven ATP synthesis.

Decreased Electron Transfer in Aging

A common observation in mammalian aging studies is the decreased electron transfer in mitochondria isolated from old animals (*3, 4*). In rodent aging studies, it is convenient to have at least three experimental groups: young animals at full adulthood (20- to 24-weeks mice or rats); old animals (48- to 56-weeks mice or rats); and senescent animals (76- to 90-weeks mice or rats). The rule of one rodent week equals to one human year is useful. Mitochondria isolated from brain, liver, heart, and kidney of old and senescent rats and mice show decreased electron transfer activity in complexes I and IV, whereas complexes II and III are largely unaffected (*4–8*) (Table 1). The decreased activity of brain complex I was observed as a decreased NADH-cytochrome c reductase activity with a simultaneously unchanged succinate-cytochrome c reductase activity in mitochondrial membranes (*4–8*), and as a decreased

Table 1
Enzyme activities of complexes I and IV and oxidative damage in whole brain mitochondria and neurological performance in aging mice

Group and age	Complex I	Complex IV	Oxidative damage	Neurological performance
Control mice, 28 week	330 ± 10	124 ± 8	5.1 ± 0.3	58–81
Control mice, 52 week	273 ± 9*	96 ± 8*	7.1 ± 0.4*	37*–54*
Control mice, 78 week	212 ± 8*	79 ± 7*	8.7 ± 0.4*	17*–45*
Moderate exercise, 52 week	291 ± 9	112 ± 8†	5.9 ± 0.3†	38–67†
Moderate exercise, 78 week	275 ± 9†	100 ± 7†	8.6 ± 0.4	30†–55†
Vitamin E, 52 week	290 ± 10	107 ± 8†	5.5 ± 0.4†	49†–73†
Vitamin E, 78 week	283 ± 9†	102 ± 7†	6.5 ± 0.4†	37†–58†
Caloric restriction, 52 week	300 ± 9†	115 ± 9	5.7 ± 0.3†	42†–70†
Caloric restriction, 78 week	286 ± 8†	110 ± 9†	5.9 ± 0.3†	31†–53†
High neurological activity, 52 week	285 ± 10	105 ± 8†	5.2 ± 0.4†	38–67†
High neurological activity, 78 week	250 ± 9†	95 ± 7†	6.9 ± 0.4†	25†–47

Young (28 week), aged (52 week) and senescent (78 week) Swiss CD1-UCadiz male mice subjected to specified life conditions from 28 week of age to 76 week of age. Data taken from refs. *2* and *6–8*. The activities of complexes I and IV are expressed in nmol cytochrome c (reduced or oxidized)/min mg protein; oxidative damage is expressed in arbitrary units as [0.1 × protein carbonyls + TBARS (both in nmol/mg protein)]/2; and neurological performance as the pair of mean values corresponding, respectively, to the percentages of successes of individual mouse in the tightrope and the T-maze tests. Twelve mice in each group.

*$P < 0.05$ for aging, compared with previous age.

†$P < 0.05$ for specific life condition compared with control mice of the same age.

mitochondrial respiratory rate in state 3 with malate-glutamate and other NAD-dependent substrates with a less diminished respiratory rate with succinate (*7, 9*) (Fig. 1). Moreover, the inhibition of complex I activity upon aging should occur with increased reduction of complex I and an increased rate of O_2^- generation (*10*).

The decreased activity of complex IV in aged mammalian brain was observed with the assay of the enzymatic activity in mitochondrial fragments (*4–8, 11*) (Table 1) and with the histochemical assay of cytochrome oxidase in human *substantia nigra* (*12*) and rat hippocampus (*13*).

Northern blot analysis of mice brain mitochondria revealed an increased expression of mitochondrial genes for complexes I, III, IV, and V in 12- and 18-month mice as compared with 2-month mice, suggesting a compensatory gene up-regulation with over-production of electron transfer proteins that is exhausted in 24-month mice (*14*).

Mitochondrial respiration could also be limited by the activities of NAD-dependent dehydrogenases but there are no consistent reports on the effects of aging in the activities of the dehydrogenases of the citric acid cycle (*4*). At variance, carnitine acyltransferase activity has been reported to decrease in aged rat brain (*15*).

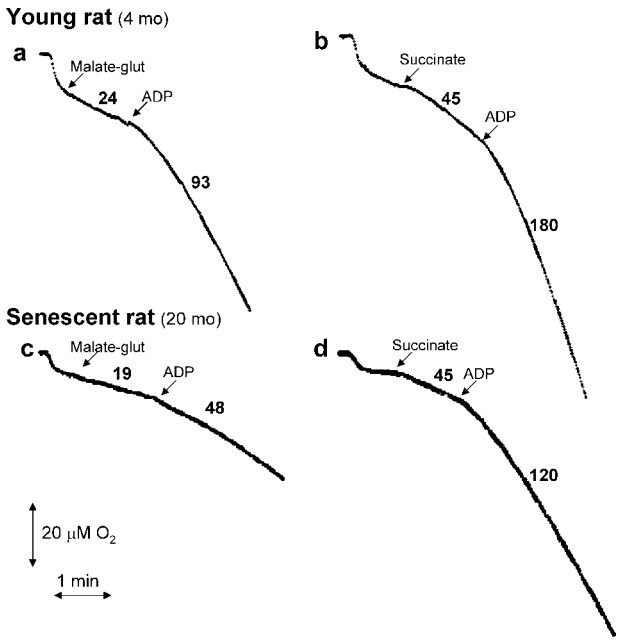

Figure 1. Hippocampal mitochondrial O_2 uptake in young and senescent rats. Respiratory rates were determined at 30 °C with 5 mM malate-5 mM glutamate or 10 mM succinate and 0.5 mM ADP. The numbers near the traces indicate respiration in ng-at O/min mg protein. a and b, mitochondria of a 4-month-old rat (1.15 mg protein/ml), c and d, mitochondria of a 20-month-old rat (1.05 mg protein/ml).

Inner Membrane H^+ Permeability, Proton Motive Potential, and H^+-Driven ATP Synthesis in Aging

There were speculations about increased H^+ permeability of the inner membrane in aging animals (*3*), but there is no evidence reported. Aged rats show decreased membrane potential in cortical and striatal mitochondria (*16*) and in whole brain mitochondria (*17*), a fact that is now interpreted as due to decreased electron transfer.

The H^+-driven ATP synthesis in aging rat brain has been estimated from the ADP/O ratios in coupled mitochondria. Slightly decreased ADP/O ratios were reported in aged brain mitochondria (*7*). There is no report on the effect of aging on the activities of the ATP-synthase or the F_1-ATPase.

DECREASED CAPACITY OF ENERGY SUPPLY IN OLD MAMMALS

The current concepts of the homeostatic control of respiration by energy demands in mammalian organs are that oxygen uptake is up-regulated by cellular ADP levels and that ATP is provided by a fraction of cell mitochondria respiring in metabolic state 3. Such fraction has been calculated for young rats under basal conditions as about 33% for liver and heart and about 50% for hippocampus (*18, 19*). In these young animals, under increased ATP demands cells can increase the rate of ATP synthesis by switching more mitochondria from resting state 4 to active state 3. In such way, young liver and heart have an upper limit of ATP production that is 2.8 times the basal rate and young hippocampus have a limit for ATP production that is 2.0 times the basal rate. Aged liver and heart, with a 30% reduction in complex I activity (*4–8*) can increase ATP production by 1.9 times by switching mitochondria from state 4 to state 3. The situation is worst in the postmitotic neurons of aged hippocampus where the decrease in electron transfer activity is more marked and mitochondria are unable to respond to any increased ATP demands (*19*).

The observed age-dependent decrease of electron transfer in brain mitochondria is simultaneous with the development of a mitochondrial subpopulation with increased size (*17*) and fragility (*5*). Most neurons are long living cells that age at the same time as the animal ages with neurogenesis spatially restricted and quantitatively negligible. An estimation of the half life ($t_{1/2}$) of mitochondrial proteins and components indicates a $t_{1/2}$ of 4–5 weeks for the turnover of brain mitochondria (*20*).

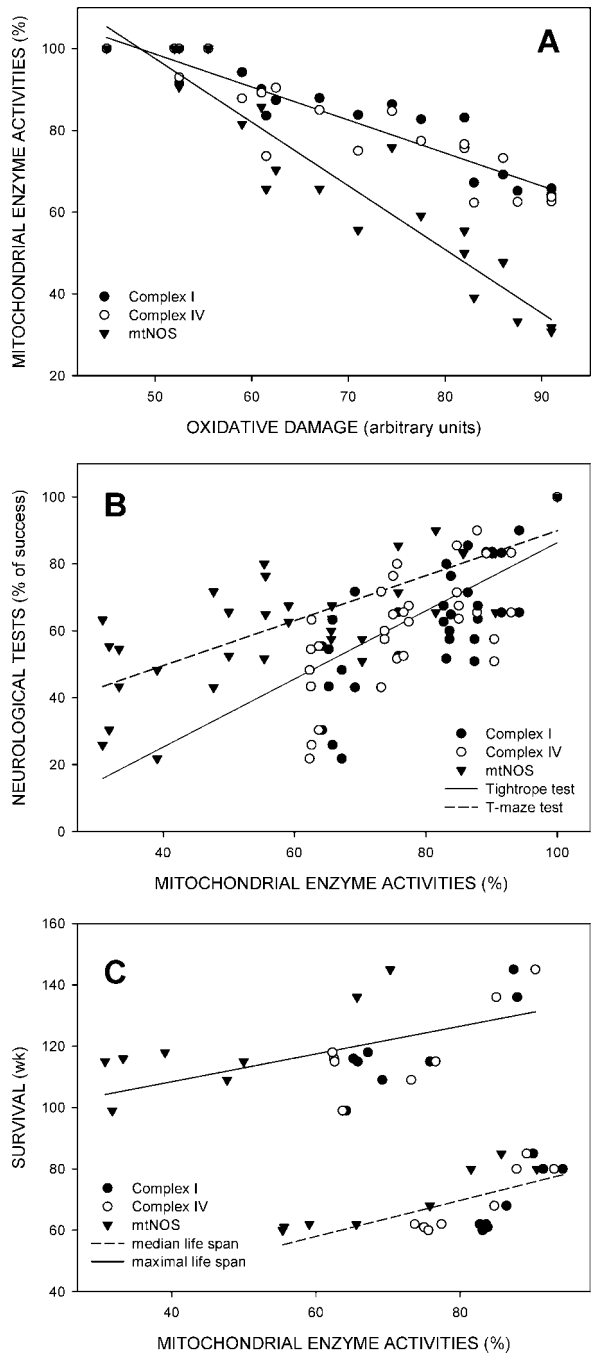

Figure 2. Statistical correlations involving: (A) mitochondrial enzyme (complexes I and IV and mtNOS) activities and mitochondrial oxidative damage, as defined in Table 1 footnote ($r^2 = 0.84$; $P < 0.01$ for complexes I and IV; $r^2 = 0.90$; $P < 0.01$ for mtNOS); (B) enzyme activities and neurological tests (tightrope, $r^2 = 0.62$; $P < 0.001$; T-maze, $r^2 = 0.56$; $P < 0.05$) and (C) enzyme activities and median ($r^2 = 0.46$; $P < 0.05$), and maximal ($r^2 = 0.31$; $P < 0.01$), life spans in male mice. Data from refs. *2* and *6–8*. For the neurological assays, individual mice were subjected every 2 week to the tightrope and the T-maze tests (*2*). For the tightrope test, mice were placed hanging from their anterior legs in the middle of a 60-cm tightrope and the test was considered successful when mice reached the column at the end of the rope in less of 30 sec. For the T-maze test, mice were challenged in a T-shaped maze of 50 cm arms and the test was considered successful when mice moved towards the T-intersection in less than 30 sec.

FREE RADICALS, OXIDATIVE DAMAGE, MITOCHONDRIAL DYSFUNCTION AND APOPTOSIS

The free radical theory of aging is based on the works of Gerschman et al. (*21*) and Harman (*22*) when focused on mitochondria, which emerges as the mitochondria hypothesis of aging (*3, 4*). Mitochondria are considered pacemakers of tissue aging due to the continuous production of oxygen and nitrogen free radicals and to the oxidative damage associated to mitochondrial dysfunction in aged mammals. A usual finding in aging is the

increased content of oxidation products of phospholipids, proteins and DNA that correspond to the free-radical mediated oxidation of cellular and mitochondrial constituents. Protein carbonyls (*4–8, 23*) and TBARS (*4–8*) (Table 1) as well as organic hydroperoxides (*17*), and 8-hydroxy-deoxyguanine (*3, 17*) have been reported to increase in brain mitochondria of aged mammals. It has been found that a combination of the mitochondrial content of protein carbonyls and TBARS affords a robust indicator of oxidative damage (*4, 19*) (Fig. 2A).

Proteomic analysis in rat cerebellum showed increased and cumulative protein nitration in aging (*24*). The activity of Mn-SOD, which is inhibited by nitration, decreased linearly in rat and mouse brain mitochondria upon aging (*2, 6*). Oxidized and nitrated proteins appear as the sand grains of a sand clock that drive mitochondrial turnover and apoptosis. The proteolytic enzymes that degrade modified proteins decline with aging, which implies a less efficient removal and an accumulation of oxidized proteins (*25*).

The role of mitochondria in the execution of the cell death program is well established (*26*). Cells with dysfunctional mitochondria are at the threshold of mitochondria-driven apoptosis. The sequence involves an initial step with increased cytosolic [Ca^{2+}] that activates mtNOS and other NOS, with increased intracellular [NO] and [H_2O_2], followed by increased lipid peroxidation, mitochondrial dysfunction, and cytochrome c release. This later leads to apotosome assembly, caspase activation, and DNA fragmentation (*26, 27*). Brain and brain areas, as the hippocampus, show an accumulation of dysfunctional mitochondria on aging, which are understood as a cause of apoptosis and tissue atrophy (*19*).

Mitochondrial Inner Membrane Enzymes that are Markers of Aging

Mitochondrial enzymes such as complex I, complex IV, mtNOS (*7*), and carnitine acyltransferase (*15*) show, about 30–60%, decreased activities in senescent rodent brain and behave as markers of aging. In mouse brain aging, mitochondrial oxidative damage, determined by the content of protein carbonyls and TBARS, negatively correlated with the enzymatic activities of complex I, complex IV, and mtNOS (Fig. 2A).

Interestingly, mice neurological function, determined by the tightrope test to evaluate neuromuscular coordination and by the T-maze test to evaluate memory and exploratory capacity, was markedly decreased upon aging (Table 1) and linearly related to brain complex I and IV enzymatic activities (*4–7*) (Fig. 2B) and negatively correlated with the mitochondrial content of brain lipid and protein oxidation products (*2, 7, 23*). Applying the concept of rate limiting step in a complex system, it follows that the decreased rates of electron transfer operate as limiting step in the energy supply in neurons and contribute to the neurological dysfunction inherent to physiological aging.

The activity of mtNOS, a constitutive inner mitochondrial membrane enzyme regulated by membrane potential (*28*), decreased significantly (*40–65%*) in the brain of senescent mice and rats (*7, 8*) as the most sensitive marker of aging (Fig. 2A). A fundamental role of NO in mitochondrial biogenesis has been reported by stimulation of guanylate cyclase, generation of cGMP, and activation of PGC-1α (*29*). Determination of the cytosolic volume occupied by mitochondria, of cytochrome oxidase activity, and of the levels of PGC-1α, nuclear regulatory factors (NRF-1, NRF-2 and TFAM) are the current experimental approaches to study the highly homeostatic process of mitochondrial biogenesis upon aging (*29, 30*).

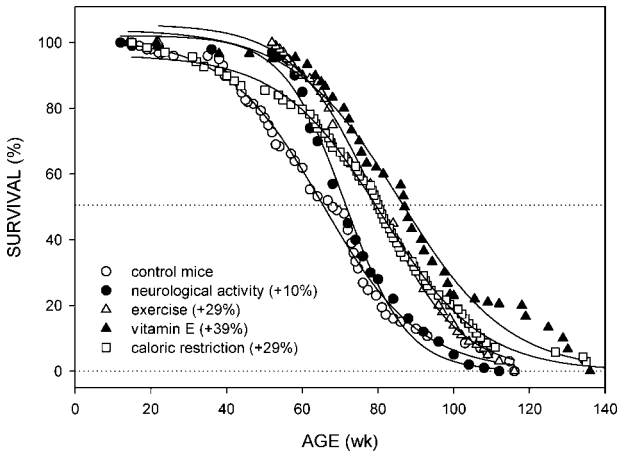

Figure 3. Effect of selected life conditions on the survival of male mice. Data from refs. *2* and *6–8*. Median life spans were: 61 ± 4 week (control); 80 ± 4 week (moderate exercise); 85 ± 4 week (vitamin E supplementation), 80 ± 3 week (caloric restriction) and 68 ± 3 week (high neurological activity). The percentages in the figure indicate the increases in median life span.

CONDITIONS THAT EXTEND MICE LIFE SPAN ALSO IMPROVE NEUROLOGICAL PERFORMANCE AND BRAIN MITOCHONDRIAL FUNCTION

It has been observed that life conditions or chronic treatments that extend the median life span in mice (Fig. 3) also improve the neurological deficits of mouse senescence and decrease the age-related oxidative damage and dysfunction of brain mitochondria, with a significant correlation between mitochondrial enzyme activities and median and maximal life span (Fig. 2C). Mice chronically subjected to moderate exercise, high doses of vitamin E, reduced food intake or with higher spontaneous neurological activity showed increased survival associated with improved neurological functions and brain mitochondrial properties (*2, 6, 7*) (Table 1).

Moderate exercise retards some physiological dysfunctions of the aging process by increasing muscle mass and strength in elderly individuals and by beneficial effects on other organs, such as brain, heart, liver, and kidney (*6*). It has been postulated that exercise triggers a β-endorphin-like response that favors mitochondrial function in brain and other organs (*31*).

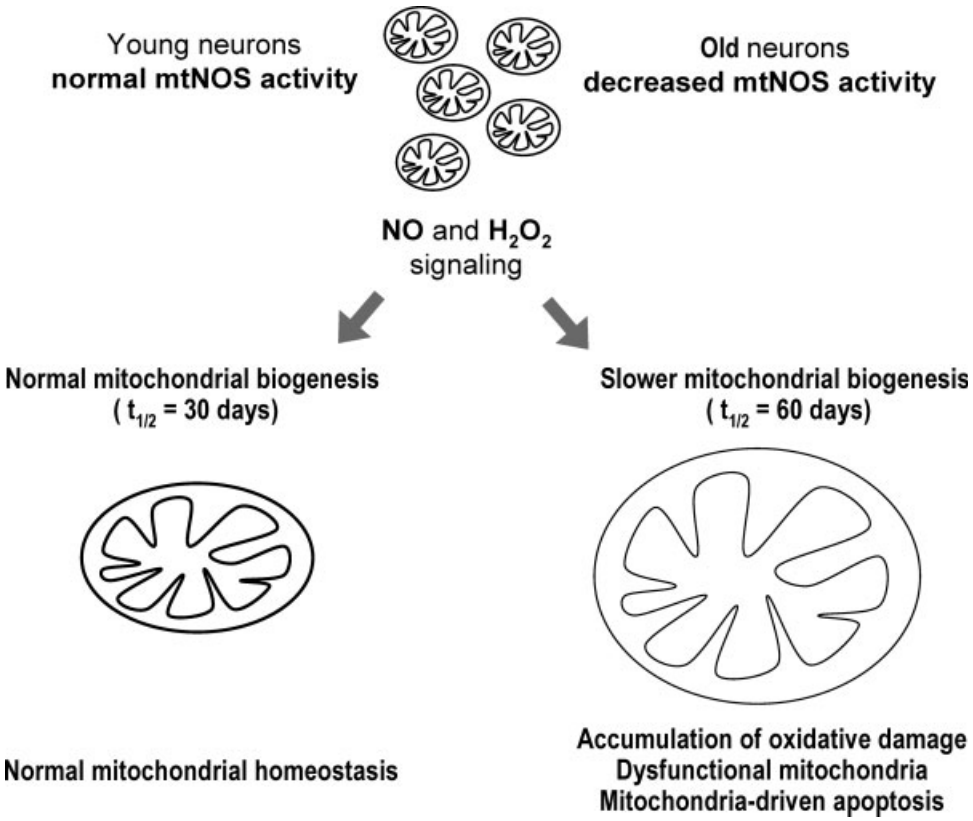

Figure 4. Scheme indicating the role of mitochondrial NO and H_2O_2 in mitochondrial biogenesis and the impairment in mitochondrial and cell homeostasis in aged brain.

Supplementation with high doses of vitamin E extended life span and improved neurological and brain mitochondrial function in aging mice (*7*). Dietary supplementation with other antioxidants, as acetylcarnitine and lipoic acid (*15, 32*) and with flavonoid-rich vegetable extracts (*17, 33*) also prevented the age-associated decline in neurological functions and oxidative damage in brain mitochondria. Rats treated chronically with acetylcarnitine showed a lower age-dependent decline in the mitochondrial oxidation rate of NAD-dependent substrates (*9*) and in the mitochondrial gene expression of complexes I, IV, and V and of adenine nucleotide translocase (*34*). The effect of high doses of vitamin E on mice survival (Fig. 3) (*7*) is to be taken into account in the controversy on the use of vitamin E supplementation in humans. The meta-analysis based claim that vitamin E supplementation increases human mortality (*35*) is challenged by the clinical evidence that vitamin E supplements are safe at high intakes (*36*) and by the reported effects of vitamins E and C in the reduction of prevalence and incidence of Alzheimer disease in an elderly population (*37*).

Limitation in food intake or caloric restriction is a well-established way to extend life span in mammals. The expectations that caloric restriction would lower basal metabolic rate were not confirmed (*38*). Growing evidence indicates that the beneficial effects of caloric restriction involve mitochondria as target subcellular structure. It has been suggested that caloric restriction provides neuroprotection by apoptosis repression (*39*) and by promotion of mitochondrial biogenesis (*40*). Thioproline, a physiological metabolite of 5-hydroxytryptamine, showed an anorexic effect with lower food intake and lower body weight accompanied by increased median life span and improved neurological and brain mitochondrial functions (*8*).

Mice with higher spontaneous neurological activity, selected by better performance on neurological tests, showed a moderately increased median life span, and improvements in behavioral tests and in mitochondrial function (*2*).

HUMAN AGING AND NEURODEGENERATIVE DISEASES

Human neurodegenerative diseases are characterized by cumulative neuronal damage that leads to neurological deficits when neuronal loss reaches a critical limit. Parkinson's disease evolves for years before typical motor signs appear with a loss of about 60% of the dopaminergic neurons of *substantia nigra pars compacta*. There is evidence that mitochondrial dysfunction and impairment of the respiratory complexes is associated with the neuronal loss of neurodegenerative diseases.

Decreased complex IV activity has been reported in Alzheimer's disease (*41*) and decreased complex I activity is usually reported in the *substantia nigra* of *postmorten* samples (*42, 43*). Inhibition of complex I create an environment of mitochondrial oxidative damage and of nitration that leads neurons to the threshold of apoptosis and to aggregation of α-synuclein with the subsequent death of dopaminergic neurons (*44*).

It is to be expected that slowing down the processes of mitochondrial dysfunction upon aging will decrease the neurological deficits in aged humans. Antioxidants targeted to mitochondria are promising therapeutic agents for human neurodegenerative diseases (*45*).

CONCLUDING REMARKS

The accumulation of dysfunctional brain mitochondria with oxidative damage, which is produced by free radical-mediated reactions, appears as a determining factor in brain aging, neurological performance, and mammalian life span. Dysfunctional mitochondria by mitochondria-dependent apoptosis drive the brain to a physiological deficit. Aged brain mitochondria show an increased content of oxidation products, an observation that puzzled researchers considering that mitochondria have a much faster turnover than neurons. The hypothesis is that aged neurons loose the capacity to synthesize mtNOS with a decrease in the NO diffusion to the cytosol and in NO-mediated mitochondrial biogenesis (Fig. 4). A mitochondrial high energy charge is characterized by high rates of NO and H_2O_2 diffusion to the cytosol (*28*). A twice slower mitochondrial turnover leads to twice increased content of oxidation products, because mitochondria continuously generate oxidation products in a linear relationship with time and respiration. The study of mitochondrial NO and H_2O_2 diffusion to the cytosol as a function of age will contribute to the understanding of mitochondrial biogenesis, cell proliferation and apoptosis in aging, a process that leads to cellular energy deficits and tissue atrophy.

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