Mitochondria, Oxidative Stress, and Pollutants including Thimerosal

1. Mitochondrial dysfunction, oxidative stress, regulation of exocytosis and their relevance to neurodegenerative diseases

Keating DJ.

A common feature in the early stages of many neurodegenerative diseases lies in mitochondrial dysfunction, oxidative stress, and reduced levels of synaptic transmission. Many genes associated with neurodegenerative diseases are now known to regulate either mitochondrial function, redox state, or the exocytosis of neurotransmitters. Mitochondria are the primary source of reactive oxygen species and ATP and control apoptosis. Mitochondria are concentrated in synapses and significant alterations to synaptic mitochondrial localization, number, morphology, or function can be detrimental to synaptic transmission. Mitochondrial by-products are capable of regulating various steps of neurotransmission and mitochondrial dysfunction and oxidative stress occur in the early stages of many neurodegenerative diseases. This mini-review will highlight the prospect that mitochondria regulates synaptic exocytosis by controlling synaptic ATP and reactive oxygen species levels and that dysfunctional exocytosis caused by mitochondrial abnormalities may be a common underlying phenomenon in the initial stages of some human neurodegenerative diseases.

PMID: 17961149

2. Mitochondria, metabolic disturbances, oxidative stress and the kynurene system, with focus on neurodegenerative disorders

Sas K et al.

The mitochondria have several important functions in the cell. A mitochondrial dysfunction causes an abatement in ATP production, oxidative damage and the induction of apoptosis, all of which are involved in the pathogenesis of numerous disorders. This review focuses on mitochondrial dysfunctions and discusses their consequences and potential roles in the pathomechanism of neurodegenerative disorders. Other pathogenetic factors are also briefly surveyed. The second part of the review deals with the kynurenine metabolic pathway, its alterations and their potential association with cellular energy impairment in certain neurodegenerative diseases. During energy production, most of the O(2) consumed by the mitochondria is reduced fully to water, but 1-2% of the O(2) is reduced incompletely to give the superoxide anion (O(2)(-)). If the function of one or more respiratory chain complexes is impaired for any reason, the enhanced production of free radicals further worsens the mitochondrial function by causing oxidative damage to macromolecules, and by opening the mitochondrial permeability transition pores thereby inducing apoptosis. These high-conductance pores offer a pathway which can open in response to certain stimuli, leading to the induction of the cells’ own suicide program. This program plays an essential role in regulating growth and development, in the differentiation of immune cells, and in the elimination of abnormal cells from the organism. Both failure and exaggeration of apoptosis in a human body can lead to disease. The increasing amount of superoxide anions can react with nitric oxide to yield the highly toxic peroxynitrite anion, which can destroy cellular macromolecules. The roles of oxidative, nitritative and nitrosative damage are discussed. Senescence is accompanied by a higher degree of reactive oxygen species production, and by diminished functions of the endoplasmic reticulum and the proteasome system, which are responsible for maintenance of the normal protein homeostasis of the cell. In the event of a dysfunction of the endoplasmic reticulum, unfolded proteins aggregate in it, forming potentially toxic deposits which tend to be resistant to degradation. Cells possess adaptive mechanisms with which to avoid the accumulation of incorrectly folded proteins. These involve molecular chaperones that fold proteins correctly, and the ubiquitin proteasome system which degrades misfolded, unwanted proteins. Both the endoplasmic reticulum and the ubiquitin proteasome system fulfill cellular protein quality control functions. The kynurenine system: Tryptophan is metabolized via several pathways, the main one being the kynurenine pathway. A central compound of the pathway is kynurenine (KYN), which can be metabolized in two separate ways: one branch furnishing kynurenic acid, and the other 3-hydroxykynurenine and quinolinic acid, the precursors of NAD. An important feature of kynurenic acid is the fact that it is one of the few known endogenous excitatory amino acid receptor blockers with a broad spectrum of antagonistic properties in supraphysiological concentrations. One of its recently confirmed sites of action is the alpha7-nicotinic acetylcholine receptor and interestingly, a more recently identified one is a higher affinity positive modulatory binding site at the AMPA receptor. Kynurenic acid has proven to be neuroprotective in several experimental settings. On the other hand, quinolinic acid is a specific agonist at the N-methyl-d-aspartate receptors, and a potent neurotoxin with an additional and marked free radical-producing property. There are a number of neurodegenerative disorders whose pathogenesis has been demonstrated to involve multiple imbalances of the kynurenine pathway metabolism. These changes may disturb normal brain function and can...
3. Mitochondria, oxidative stress and cell death

Ott M et al.

In addition to the well-established role of the mitochondria in energy metabolism, regulation of cell death has recently emerged as a second major function of these organelles. This, in turn, seems to be intimately linked to their role as the major intracellular source of reactive oxygen species (ROS), which are mainly generated at Complex I and III of the respiratory chain. Excessive ROS production can lead to the oxidation of macromolecules and has been implicated in mtDNA mutations, ageing, and cell death. Mitochondria-generated ROS play an important role in the release of cytochrome c and other pro-apoptotic proteins, which can trigger caspase activation and apoptosis. Cytochrome c release occurs by a two-step process that is initiated by the dissociation of the hemoprotein from its binding to cardiolipin, which anchors it to the inner mitochondrial membrane. Oxidation of cardiolipin reduces cytochrome c binding and results in an increased level of "free" cytochrome c in the intermembrane space. Conversely, mitochondrial antioxidant enzymes protect from apoptosis. Hence, there is accumulating evidence supporting a direct link between mitochondria, oxidative stress and cell death.

PMID: 17453160

4. Oxidative stress and mitochondrial dysfunction in neurodegenerative diseases

Trushina E, McMurray CT.

In recent years, it has become increasingly clear that mitochondrial dysfunction and oxidative damage are major contributors to neuronal loss. Free radicals, typically generated from mitochondrial respiration, cause oxidative damage of nucleic acids, lipids, carbohydrates and proteins. Despite enormous amount of effort, however, the mechanism by which oxidative damage causes neuronal death is not well understood. Emerging data from a number of neurodegenerative diseases suggest that there may be common features of toxicity that are related to oxidative damage. In this review, while focusing on Huntington's disease (HD), we discuss similarities among HD, Friedreich ataxia and xeroderma pigmentosum, which provide insight into shared mechanisms of neuronal death.

PMID: 17303344

5. Lipoic acid and N-acetyl cysteine decrease mitochondrial-related oxidative stress in Alzheimer disease patient fibroblasts

Moreira PI et al.

In this study, we evaluated the effect of lipoic acid (LA) and N-acetyl cysteine (NAC) on oxidative [4-hydroxy-2-nonenal, N(epsilon)-(carboxymethyl)lysine and heme oxygenase-1] and apoptotic (caspase 9 and Bax) markers in fibroblasts from patients with Alzheimer disease (AD) and age-matched and young controls. AD fibroblasts showed the highest levels of oxidative stress, and the antioxidants, lipoic acid (1 mM) and/or N-acetyl cysteine (100 microM) exerted a protective effect as evidenced by decreases in oxidative stress and apoptotic markers. Furthermore, we observed that the protective effect of LA and NAC was more pronounced when both agents were present simultaneously. AD-type changes could be generated in control fibroblasts using N-methylprotoporphyrin to inhibit cytochrome oxidase assembly indicating that the oxidative damage observed was associated with mitochondrial dysfunction. The effects of N-methylprotoporphyrine were reversed or attenuated by both lipoic acid and N-acetyl cysteine. These data suggest mitochondria are important in oxidative damage that occurs in AD. As such, antioxidant therapies based on lipoic acid and N-acetyl cysteine supplementation may be promising.

PMID: 17917164
Mitochondria and thimerosal

6: Thimerosal induces neuronal cell apoptosis by causing cytochrome c and apoptosis-inducing factor release from mitochondria

Yel L et al.

There is a worldwide increasing concern over the neurological risks of thimerosal (ethylmercury thiosalicylate) which is an organic mercury compound that is commonly used as an antimicrobial preservative. In this study, we show that thimerosal, at nanomolar concentrations, induces neuronal cell death through the mitochondrial pathway. Thimerosal, in a concentration- and time-dependent manner, decreased cell viability as assessed by calcein-ethidium staining and caused apoptosis detected by Hoechst 33258 dye. Thimerosal-induced apoptosis was associated with depolarization of mitochondrial membrane, generation of reactive oxygen species, and release of cytochrome c and apoptosis-inducing factor (AIF) from mitochondria to cytosol. Although thimerosal did not affect cellular expression of Bax at the protein level, we observed translocation of Bax from cytosol to mitochondria. Finally, caspase-9 and caspase-3 were activated in the absence of caspase-8 activation. Our data suggest that thimerosal causes apoptosis in neuroblastoma cells by changing the mitochondrial microenvironment.

PMID: 16273274

7: Mitochondrial mediated thimerosal-induced apoptosis in a human neuroblastoma cell line (SK-N-SH)

Humphrey ML et al.

Environmental exposure to mercurials continues to be a public health issue due to their deleterious effects on immune, renal and neurological function. Recently the safety of thimerosal, an ethyl mercury-containing preservative used in vaccines, has been questioned due to exposure of infants during immunization. Mercurials have been reported to cause apoptosis in cultured neurons; however, the signaling pathways resulting in cell death have not been well characterized. Therefore, the objective of this study was to identify the mode of cell death in an in vitro model of thimerosal-induced neurotoxicity, and more specifically, to elucidate signaling pathways which might serve as pharmacological targets. Within 2 h of thimerosal exposure (5 microM) to the human neuroblastoma cell line, SK-N-SH, morphological changes, including membrane alterations and cell shrinkage, were observed. Cell viability, assessed by measurement of lactate dehydrogenase (LDH) activity in the medium, as well as the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, showed a time- and concentration-dependent decrease in cell survival upon thimerosal exposure. In cells treated for 24 h with thimerosal, fluorescence microscopy indicated cells undergoing both apoptosis and oncosis/necrosis. To identify the apoptotic pathway associated with thimerosal-mediated cell death, we first evaluated the mitochondrial cascade, as both inorganic and organic mercurials have been reported to accumulate in the organelle. Cytochrome c was shown to leak from the mitochondria, followed by caspase 9 cleavage within 8 h of treatment. In addition, poly(ADP-ribose) polymerase (PARP) was cleaved to form a 85 kDa fragment following maximal caspase 3 activation at 24 h. Taken together these findings suggest deleterious effects on the cytoarchitecture by thimerosal and initiation of mitochondrial-mediated apoptosis.

PMID: 15869795

8. Biochemical and molecular basis of thimerosal-induced apoptosis in T cells: a major role of mitochondrial pathway

Makani S et al.

The major source of thimerosal (ethyl mercury thiosalicylate) exposure is childhood vaccines. It is believed that the children are exposed to significant accumulative dosage of thimerosal during the first 2 years of life via immunization. Because of health-related concerns for exposure to mercury, we examined the effects of thimerosal
on the biochemical and molecular steps of mitochondrial pathway of apoptosis in Jurkat T cells. Thimerosal and
not thiosalicylic acid (non-mercury component of thimerosal), in a concentration-dependent manner, induced
apoptosis in T cells as determined by TUNEL and propidium iodide assays, suggesting a role of mercury in T cell
apoptosis. Apoptosis was associated with depolarization of mitochondrial membrane, release of cytochrome c
and apoptosis inducing factor (AIF) from the mitochondria, and activation of caspase-9 and caspase-3, but not of
caspase-8. In addition, thimerosal in a concentration-dependent manner inhibited the expression of XIAP, cIAP-1
but did not influence cIAP-2 expression. Furthermore, thimerosal enhanced intracellular reactive oxygen species
and reduced intracellular glutathione (GSH). Finally, exogenous glutathione protected T cells from thimerosal-
induced apoptosis by upregulation of XIAP and cIAP1 and by inhibiting activation of both caspase-9 and caspase-
3. These data suggest that thimerosal induces apoptosis in T cells via mitochondrial pathway by inducing
oxidative stress and depletion of GSH.

PMID: 12140745

9: Inhibition of malate transport and activation of phosphate transport in mitochondria by
ethylmercurithiosalicylate
Freitag H, Kadenbach B.
PMID: 7409159

10: Ethylmercurithiosalicylate--a new reagent for the study of phosphate transport in mitochondria.
Freitag H, Kadenbach B.
PMID: 7389907

Mitochondria and other pollutants

Comment: Windham et al 2006 identified several airborne pollutants associated with autism, including "mercury,
cadmium, nickel, trichloroethylene, and vinyl chloride" (20). Other pollutants appear in urinary and fetal
evaluations. Many pollutants adversely affect mitochondria.

11: Autism spectrum disorders in relation to distribution of hazardous air pollutants in the san francisco
bay area
Windham GC et al.
Environ Health Perspect. 2006 Sep;114(9):1438-44.

12: Induction of reactive oxygen species by bisphenol A and abrogation of bisphenol A-induced cell
injury by DJ-1
Ooe H et al.
http://toxsci.oxfordjournals.org/cgi/content/full/88/1/114

DJ-1 was first identified as an activated ras-dependent oncogene. DJ-1 is related to male fertility, and its
expression in sperm decreases in response to exposure to a number of reproductive toxicants. DJ-1 has been
associated with the onset of familial Parkinson's disease (PD) in humans, and has been found to have activity
against oxidative damage by eliminating reactive oxygen species (ROS). In this study, we investigated the role of
DJ-1 in oxidative stresses by administration of bisphenol A (BPA), which has been reported to induce oxidative
stress in rodents, to male mice and cultured cells. In male mice, we found that BPA significantly increased the
expression level of DJ-1 in the sperm and brain. In cultured Neuro2a and GC1 cells, we found that BPA induced
ROS production and significantly compromised mitochondrial function concomitant with elevated expression and
oxidization of DJ-1. DJ-1 was found to maintain the complex I activity against BPA-induced oxidative stress after
the localization in mitochondria. The results showed that DJ-1 plays a role in the prevention of mitochondrial
injury-induced cell death.

PMID: 16093527
13. Thallium induces hydrogen peroxide generation by impairing mitochondrial function


Thallium (Tl) is highly toxic through yet poorly understood mechanisms. In this study, we comparatively investigated the effects of thallic (Tl(III)) cations on mitochondrial functionality and oxidative stress promotion, and results were compared to those obtained for thallous (Tl(I)) cation. PC12 cells were incubated between 1 and 72 h in the presence of a single dose of Tl(I) or Tl(III) (10-250 microM). A metal concentration- and time-dependent decrease in cell viability was observed evaluated by both MTT reduction and calcein fluorescence. After 24 h in culture, Tl(I) and Tl(III) significantly decreased mitochondrial membrane potential evaluated as the incorporation of rhodamine 123. Along the incubation period assessed, both Tl(I) and Tl(III) (50 and 100 microM) significantly increased mitochondrial H2O2 steady-state levels, being the magnitude of the effect: Tl(III)>Tl(I). Glutathione content, measured by reaction with monochlorobimane, was significantly reduced in Tl-treated cells. Finally, higher oxidant species content in cells cytoplasm was found, which positively correlated with mitochondrial H2O2 content. Together, these results indicate that both ionic species of Tl enhance cells reactive oxygen species production, decreasing mitochondrial functionality. These effects could partially be responsible for the loss of cell viability, and account for the metabolic alterations found in Tl intoxication.

PMID: 16934846

14. Cadmium induced mitochondrial injury and apoptosis in vero cells: protective effect of diallyl tetrasulfide from garlic


Oxidative stress and mitochondrial injury has been implicated in cadmium-induced apoptosis. In this study, we examined the protective effect of diallyl tetrasulfide from garlic on cadmium induced oxidative stress and apoptosis in vero cells. Exposure of vero cells to cadmium (10 microM) for 18 h showed the apoptotic events such as loss of cell viability, alterations in nuclear morphology and decreased mitochondrial membrane potential with significantly increased levels of reactive oxygen species (superoxide anion and hydrogen peroxide). Treatment of vero cells with cadmium (10 microM) and diallyl tetrasulfide (5-50 microg/ml) showed that diallyl tetrasulfide attenuated the cadmium-induced suppression of cell viability in a dose dependent manner and highly significant effect was observed at 40 microg/ml. The nuclei morphological analysis with 4',6-diamidino-2-phenylindole staining confirmed that diallyl tetrasulfide at 40 microg/ml prevented the Cd (10 microM) induced apoptosis. Flow cytometric analysis with 2',7'-dichlorofluorocinc diacetate showed that the inhibitory effect of diallyl tetrasulfide (10-40 microg/ml) on reactive oxygen species generation parallel with its effect on cell viability. In addition, diallyl tetrasulfide (40 microg/ml) remarkably reduced the cadmium-induced accumulation of superoxide radical and hydrogen peroxide with in cells. Further, diallyl tetrasulfide significantly protected the cadmium-induced decrease in mitochondrial membrane potential, an indicator of mitochondrial function. Our study suggest that diallyl tetrasulfide affect the reactive oxygen species generation induced by cadmium, and possesses a novel protective effect on the cytotoxicity associated with mitochondrial injury, which contributes to the antiapoptotic effect of diallyl tetrasulfide against cadmium.

PMID: 16971165

15. Cadmium induces mitochondria-dependent apoptosis of normal human hepatocytes


The heavy metal cadmium, an environmental pollutant, has been widely demonstrated to be toxic, in particular for liver. In murines, cadmium induces apoptosis of hepatocytes and hepatomas. In human cells, apoptosis induced by cadmium has been exclusively demonstrated in tumoral cell lines. Nothing was known in normal liver, in vitro or in vivo. In the present study, we examined the effects of cadmium in nonmalignant human hepatocytes. For that purpose, we investigated whether cadmium was able to induce apoptosis of normal human hepatocytes (NHH) in primary culture and of a SV40-immortalized human hepatocyte (IH) cell line. Treatment of IH and NHH with cadmium induced the presence of a sub-G(1) population at 10 and 100 mumol/L, respectively. DAPI staining of both cell types treated with cadmium 100 mumol/L revealed the induction of nuclear apoptotic bodies, supporting the hypothesis of apoptosis. In IH and NHH, cadmium 100 mumol/L induced PARP cleavage into a
85 kDa fragment. In order to investigate the involvement of mitochondria in cadmium-induced apoptosis, we measured the mitochondrial membrane potential (ΔΨm). We observed that in IHH and NHH, cadmium 100 μmol/L induced a decrease of ΔΨm. As expected, cadmium under the same conditions enhanced caspase-9 and caspase-3 activities. In addition, cadmium from 1 to 100 μmol/L induced the expression of p53 and phosphorylation of its Ser15 in IHH and NHH. In conclusion, we showed in this study that human hepatocytes were sensitive to cadmium and apoptosis induced at concentrations suggested in the literature to inhibit p53 DNA-binding and DNA repair.

PMID: 17610031

16. Trichloroethylene: Parkinsonism and complex 1 mitochondrial neurotoxicity

Gash DM et al.

OBJECTIVE: To analyze a cluster of 30 industrial coworkers with Parkinson's disease and parkinsonism subjected to long-term (8-33 years) chronic exposure to trichloroethylene. METHODS: Neurological evaluations were conducted on the 30 coworkers, including a general physical and neurological examination and the Unified Parkinson's Disease Rating Scale. In addition, fine motor speed was quantified and an occupational history survey was administered. Next, animal studies were conducted to determine whether trichloroethylene exposure is neurotoxic to the nigrostriatal dopamine system that degenerates in Parkinson's disease. The experiments specifically analyzed complex 1 mitochondrial neurotoxicity because this is a mechanism of action of other known environmental dopaminergic neurotoxins. RESULTS: The three workers with workstations adjacent to the trichloroethylene source and subjected to chronic inhalation and dermal exposure from handling trichloroethylene-soaked metal parts had Parkinson's disease. Coworkers more distant from the trichloroethylene source, receiving chronic respiratory exposure, displayed many features of parkinsonism, including significant motor slowing. Neurotoxic actions of trichloroethylene were demonstrated in accompanying animal studies showing that oral administration of trichloroethylene for 6 weeks instigated selective complex 1 mitochondrial impairment in the midbrain with concomitant striatonigral fiber degeneration and loss of dopamine neurons. INTERPRETATION: Trichloroethylene, used extensively in industry and the military and a common environmental contaminant, joins other mitochondrial neurotoxins, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and some pesticides, as a risk factor for parkinsonism.

PMID: 18157908

17. Arsenic-induced mitochondrial instability leading to programmed cell death in the exposed individuals

Banerjee N et al.
Toxicology. 2008 Jan 12 [Epub ahead of print]

In West Bengal, India, more than 6 million people in nine districts are exposed to arsenic through drinking water. It is regarded as the greatest arsenic calamity in the world. Arsenic is a well-documented human carcinogen, which does not induce cancer in any other animal model. Interestingly, at lower concentrations, arsenic is known to induce apoptosis in various cancer cell lines in vitro. We have studied apoptosis in human peripheral blood mononuclear cells (PBMC) of 30 arsenic exposed skin lesion individuals by annexin V-FITC staining and compared with 28 unexposed individuals. The percentage of apoptotic cells in individuals with skin lesions was significantly higher (p<0.001) in comparison to unexposed individuals. In the exposed individuals with skin lesions, there were elevated levels of intracellular reactive oxygen species (ROS), mitochondrial membrane permeability and increased cytochrome c release, leading to increased downstream caspase activity. Arsenic-induced DNA damage was confirmed by DNA ladder formation and confocal microscopy. We also observed that chronic arsenic exposure reduced Bcl-2/Bax ratio and also resulted in cell cycle arrest of PBMC in G(0)/G(1) phase. All these observations indicate that mitochondria-mediated pathway may be responsible for arsenic-induced apoptosis.

PMID: 18304716

18. Arsenic induced mitochondrial DNA damage and altered mitochondrial oxidative function: implications for genotoxic mechanisms in mammalian cells

Partridge MA et al.
Cancer Res. 2007 Jun 1;67(11):5239-47.

Arsenic is a well-established human carcinogen that is chronically consumed in drinking water by millions of
people worldwide. Recent evidence has suggested that arsenic is a genotoxic carcinogen. Furthermore, we have shown that mitochondria mediate the mutagenic effects of arsenic in mammalian cells, as arsenic did not induce nuclear mutations in mitochondrial DNA (mtDNA)-depleted cells. Using the human-hamster hybrid A(L) cells, we show here that arsenic alters mitochondrial function by decreasing cytochrome c oxidase function and oxygen consumption but increasing citrate synthase function. These alterations correlated with depletion in mtDNA copy number and increase in large heteroplasmic mtDNA deletions. In addition, mtDNA isolated periodically from cultures treated continuously with arsenic did not consistently display the same deletion pattern, indicating that the mitochondrial genome was subjected to repeated and continuous damage. These data support the theory that the mitochondria, and particularly mtDNA, are important targets of the mutagenic effects of arsenic in mammalian cells.

PMID: 17545603

19. Arsenic induces apoptosis in mouse liver is mitochondria dependent and is abrogated by N-acetylcysteine
Santra A et al.

Arsenicosis, caused by arsenic contamination of drinking water supplies, is a major public health problem in India and Bangladesh. Chronic liver disease, often with portal hypertension occurs in chronic arsenicosis, contributes to the morbidity and mortality. The early cellular events that initiate liver cell injury due to arsenicosis have not been studied. Our aim was to identify the possible mechanisms related to arsenic-induced liver injury in mice. Liver injury was induced in mice by arsenic treatment. The liver was used for mitochondrial oxidative stress, mitochondrial permeability transition (MPT). Evidence of apoptosis was sought by TUNEL test, caspase assay and histology. Pretreatment with N-acetyl-L-cysteine (NAC) was done to modulate hepatic GSH level. Arsenic treatment in mice caused liver injury associated with increased oxidative stress in liver mitochondria and alteration of MPT. Altered MPT facilitated cytochrome c release in the cytosol, activation of caspase 9 and caspase 3 activities and apoptotic cell death. Pretreatment of NAC to arsenic-treated mice abrogated all these alteration suggesting a glutathione (GSH)-dependent mechanism. Oxidative stress in mitochondria and inappropriate MPT are important in the pathogenesis of arsenic induced apoptotic liver cell injury. The phenomenon is GSH dependent and supplementation of NAC might have beneficial effects.

PMID: 17303202